Characterization of the Relationship Between Whole Blood and Plasma Topiramate Concentrations

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ABSTRACT

The overall goal of my thesis was to determine the feasibility of dried blood spots (DBS) technology for measurement of topiramate (TPM) concentration and characterization of the relationship between whole blood and plasma TPM concentrations. Topiramate is widely used in the treatment of epilepsy and migraines and has shown to be effective in controlling seizures in children and adults. Topiramate has the potential to be a good therapy for neonatal seizures and Dravet syndrome. One major hurdle in developing drugs for treatment of childhood seizure disorders is the severe restriction on blood sampling in critically ill children, in whom blood is collected for a multitude of lab tests. This circumstance reduces the blood volume available for pharmacokinetics (PK) studies. Consequently, the necessary research on PK and dose finding is often significantly limited.

The relationship between whole blood and plasma TPM concentrations were explored in canines with naturally-occurring epilepsy and adult and children on maintenance TPM therapy. In addition, the PK was described in both of these populations. In the canine study, four dogs were administered both intravenous (IV) and oral topiramate with the goal of comparing the PK of IV TPM in plasma and whole blood and describing the relationship between whole blood and plasma TPM concentrations. Similar to humans, the study showed a nonlinear relationship between plasma and whole blood TPM concentration in which whole blood TPM concentrations were 1.3-3.7 times greater than plasma concentrations. In addition, there was a difference in PK parameter estimates

with those derived from whole blood generally showing reduced clearances resulting in longer elimination half-lives.

In the adult and children study, thirty adult and eight children participants currently taking TPM therapy were recruited. These studies provide previously unreported information about TPM. Whole blood concentrations were found to be different than plasma concentrations with the whole blood TPM concentrations 0.9-4.4 times greater than plasma concentrations. These are the first studies comparing plasma and DBS TPM concentration obtained from patient samples using a formula that includes both hematocrit and the analyte-specific red blood cell-to-plasma ratio. The calculated TPM plasma concentration based on whole blood and DBS concentrations are in good agreement with analyzed plasma concentrations. Additionally, the PK of TPM was found to be similar to previous reported literature values.

This thesis describes the development of bioanalytical assays to the implementation of these assays in clinical practice and clinical pharmacologic aspects of topiramate PK. Results from the studies described in this thesis provide a tool to estimate plasma concentrations from whole blood or DBS TPM concentrations. This will allow therapeutic drug monitoring using DBS and permit PK studies in populations where blood volume is limited.

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CHAPTER 1: INTRODUCTION TO CHILDHOOD EPILEPSIES

1.1. EPILEPSY

The International League Against Epilepsy (ILAE) defines epilepsy as a condition characterized by recurrent (two or more) unprovoked seizures separated by more than 24 hours and an alteration in the brain that increases the likelihood of future seizures.[1] A seizure is the clinical manifestation of an abnormal and excessive activity of a set of cortical neurons.

The focus of the research presented in this thesis involves understanding the relationship between whole blood and plasma TPM concentrations. The following two chapters will summarize pediatric epilepsy syndromes with an emphasis on disorders that use or have potential use of TPM and development pharmacokinetics and pharmacotherapy in children as it relates to these disorders.

Table 1: Operational Clinical Definition of Epilepsy[1]

Epilepsy is a disease of the brain defined by any of the following conditions
1. At least two unprovoked seizures occurring > 24 h apart 2. One unprovoked seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years 3. Diagnosis of an epilepsy syndrome
Epilepsy is considered to be resolved for individuals who had an age-dependent epilepsy syndrome but are now past the applicable age or those who have remained seizure-free for the last 10 years, with no seizure medicine for the last 5 years

1.2. EPIDEMIOLOGY

When considered a group, epilepsy disorders are among the most frequently occurring neurologic conditions in children.[2] In the United States approximately 5% of children and adolescents experience a seizure of some type by the age of 20.[2] Incidence rates for

epilepsy have been estimated over the past several years and have been found to range from 35 to 124 per 100,000 in different countries.[2] Numerous studies have discussed the prevalence of epilepsy worldwide. The definitions of prevalence vary, however, if prevalence is defined as seizures or medication in the past 5 years, estimates remain between 3 and 7 per 1,000 in developed countries and in the range of 9 to 22 per 1,000 in developing countries.[2]

1.3. CLASSIFICATION OF EPILEPTIC SEIZURES

A classification of epileptic seizures provides guidance in determining the diagnostic evaluation, treatment, and prognosis. In 2010 the ILAE proposed a revision of the terminology and concepts for organization of seizures.[3] The new terminology will be used.

1.3.1. Focal Seizures

Focal seizures arise in specific loci in the cortex within networks limited to one hemisphere. They can be classified further into those without impairment of consciousness or awareness and those with impairment of consciousness or awareness. In patients not demonstrating impairment of consciousness or awareness, focal seizures is further subdivided into seizures with: (i) observable motor or autonomic components and (ii) subjective sensory or psychic phenomenon.[4] The signs and symptoms of focal seizures depend on the location of the seizure. Seizures involving the motor cortex most commonly consist of rhythmic clonic activity of the face, arm, or leg. Seizures with

somatosensory, autonomic, and psychic symptoms may involve hallucinations, illusions, and déjà vu.

Focal seizures with impairment of consciousness are one of the most common seizure types encountered in both children and adults.[2] Focal seizures without impairment of consciousness may evolve into seizures with impaired consciousness. The beginning of the focal seizure may serve as a warning to the patient (i.e. aura) that a more severe seizure is pending. The impairment of awareness may be subtle. The patient may either not respond to commands or respond in an abnormally slow manner. Behavior is usually more complex during the seizure. Automatisms, of which the patient is unaware and subsequently cannot recall, are common during the period of impaired consciousness. Types of automatism behaviors are variable and may consist of activities such as facial grimacing, gestures, chewing, lip smacking, snapping fingers, and repeating phases.

Although variable, focal seizures usually last from 30 seconds to several minutes.[4] Most patients have some degree of postictal impairment, such as tiredness or confusion following a focal seizure.

1.3.2. Generalized Seizures

Generalized seizures involve large areas of the brain and are usually bilateral in their initial manifestations and associated with early impairment of consciousness. These can range from absence seizures, characterized only by impaired consciousness, to

generalized tonic-clonic seizures (GTC), in which widespread convulsive activity takes place.

Generalized Tonic-Clonic Seizures

GTC seizures are characterized by loss of consciousness that occurs simultaneously with the onset of a generalized stiffening of flexor or extensor muscle (termed tonic phase). Following the tonic phase, generalized jerking of the muscles (clonic activity) occurs.

Absence Seizures

Absence seizures are generalized seizures, indicating bihemispheric initial involvement clinically and electroencephalographically. Absence seizures have an abrupt onset and offset. There is typically a sudden cessation of activities with a blank, distant look to the face. As the seizure continues, there are often automatism and mild clonic motor activity such as jerks of the arms and eye blinking. The two major divisions of absence seizures are typical and atypical.

Simple atypical absence consists of the sudden onset of impaired consciousness, usually associated with a “blank” or “distant” facial appearance without other motor or behavioral phenomena. The complex typical absence is accompanied by other motor, behavioral, or autonomic phenomena. Atypical absence seizures are characterized as having more pronounced change in tone and longer duration than typical seizures.

Clonic Seizures

Clonic seizures have rhythmic or semirhythmic contractions of a group of muscles. These jerks can involve any muscle group although the arms, neck, and facial muscles are most commonly involved. Clonic seizures are more common in children than adults.[4]

Myoclonic Seizures

Myoclonic seizures are characterized by sudden, brief (<350 milliseconds), shock-like contraction that may be generalized or confined to the face and trunk or to one or more extremities. Myoclonic seizures result in short bursts of synchronized electromyographic activity which often involves simultaneous activation of agonist and antagonist muscles. The muscle contractions are quicker than the contractions with clonic seizures. These seizures may be dramatic, causing the patient to fall to the ground or may be quite subtle, resembling tremors. Because of the brevity of the seizures, it is not possible to determine if consciousness is impaired.

Tonic Seizures

Tonic seizures are brief seizures (usually less than 60 seconds) consisting of the sudden onset of increased tone in extensor muscles. If standing, the patient typically falls to the ground. The seizures are invariably longer than myoclonic seizures. There is impairment of consciousness during the seizure, although in short seizures this may be difficult to assess.

Atonic Seizures

Atonic seizures or drop attacks are characterized by a sudden loss of muscle tone. They begin without warning and cause the patient, if standing, to fall quickly to the floor. Since there may be a total loss of tone, the child has no means to protect himself and injuries often occur. Consciousness is impaired during the fall, although the patient may regain alertness immediately upon hitting the floor. Atonic attacks are frequently associated with myoclonic jerks either before, during, or after the atonic seizure.[4]

1.4. EPILEPSY SYNDROMES

There are a number of age-related syndromes which have been discussed in detail.[2,4] For this introduction, a summary of the epidemiology, etiology, and clinical course for selective childhood epilepsies will be described. A detailed discussion of all syndromes is beyond the scope of this introduction and can be found in “Pediatric Epilepsy” and “Pediatric epilepsy: diagnosis and therapy”.[2,4]

1.4.1. Newborns

Neonatal Seizures

The risk of seizures in newborns is significant and diagnosis for this disease is often hard because seizures are difficult to detect in newborns.

Epidemiology and Etiology

Neonatal seizures are defined as seizures occurring during the first 30 days of life. The incidence of seizures in children is significantly higher than in adults with the greatest

risk being in the neonatal period. Neonatal seizures are estimated to occur in 0.01% of live births (1-5/1000 live births).[5,6] This estimate has varied from 0.5% in term babies to 22.2% in preterm babies.[5,7-9]

Hypoxic ischemic encephalopathy (HIE) is the most common cause of neonatal seizures.[10,11] HIE is characterized by oxygen deprivation to the brain. This is commonly due to brain injury from asphyxia. HIE is presumed to be the cause in 50% to 60% of neonatal seizure cases.[10,11] A range of factors can also increase the risk of neonatal seizures which include inborn errors of metabolism, intracranial hemorrhaging, infections, and metabolic irregularities. In a study of 200 neonates, etiology was identified in 99% of cases. Birth asphyxia was the most common (35%) followed by CNS infections (34%), metabolic abnormalities (12.5%), and intracranial bleeding (9.5%).[12] In addition gestational age is suggested to play a role in the risk for developing neonatal seizures. The risk of seizures increases in preterm infants and infant with low birth weight.[10,13]

Pathophysiology

The early postnatal period represents a critical developmental window in which synaptogenesis is ongoing and neuronal plasticity is increased compared to adults. Excitatory synaptic transmission mediated by glutamate receptors is required for these processes and is enhanced in the immature brain.[14] Glutamate, a major excitatory neurotransmitter, has been shown to be important in the development of plasticity. The synapse with receptor localization is illustrated in Figure 1.

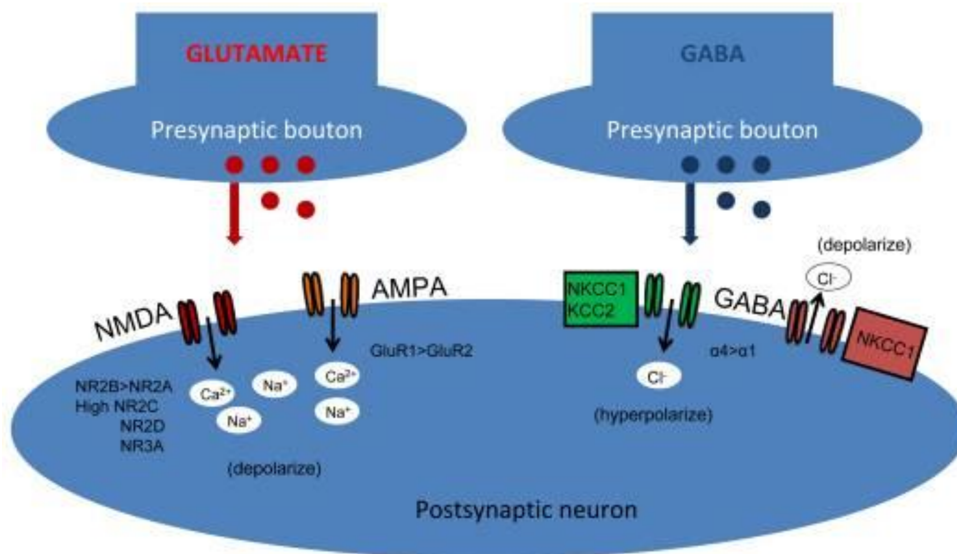


Figure 1: Dynamics of Synaptic Transmission at Cortical Synapses in the Neonatal Period[15]

Glutamate receptors (GluRs) are transiently overexpressed during development (Figure 2).[16] There are two types of GluRs, ionotropic and metabotropic. Ionotropic GluRs are ligand-gated ion channels that permit the flux of sodium, potassium, and sometimes calcium ions.[14] The main subtypes of these receptors include the *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and kainate receptors.

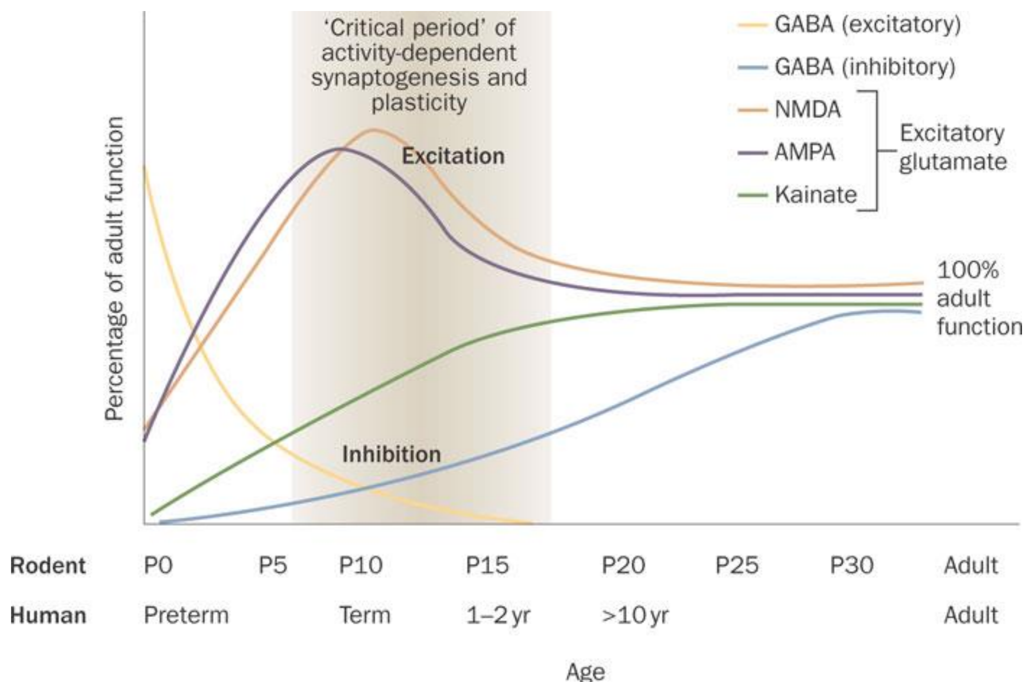


Figure 2: Schematic depiction of maturational changes in glutamate and GABA receptor function in the developing brain[17]

NMDA receptors are heteromeric, including an obligate NR1 subunit, and their makeup is developmentally regulated.[16] In the neonatal brain, the predominant NR2 subunit is the NR2B and functionally results in a longer current decay time compared to the adult NR2A subunit.[16] Other subunits, NR2C, NR2D, and NR3A, have decreased magnesium sensitivity causing increased receptor excitability.[18] Even though this receptor presents an age-specific therapeutic target, a barrier in drug development is that it is essential for learning, memory, and brain development.[16]

The AMPA glutamate receptors are suggested to be involved in fast excitatory synaptic transmission. These receptors are hetreomeric and composed of four subunits (GluR1, GluR2, GluR3, or GluR4). In the immature brain, AMPA receptors lack the GluR2

subunit compared to the adult brain, which leads to increased calcium influx.[19] This AMPA receptor composition enhances receptor excitability during the neonatal period.

The developmental regulation of metabotropic glutamate receptors is not well known. The metabotropic glutamate receptor subunits are mGluR1 through mGluR8 and are divided into three groups based on receptor structure and physiological activity (Group I: mGluR1 and mGluR5, Group II: mGluR2 and mGluR3, Group III: mGluR4, mGluR6, mGluR7, and mGluR8). Recently a study in rats showed that mGluR1 expression might peak in the first postnatal week, whereas mGluR2, mGluR3, and mGluR5 levels mature by the second postnatal week.[20] Structural features of metabotropic GluRs may offer new targets for anticonvulsants. Specifically, group II and group III metabotropic GluR agonists have anticonvulsant properties.[20]

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain. The expression and function of GABA receptors are developmentally regulated and may play an influence in neonatal seizures. Binding and expression of the GABA receptor are both lower in the immature brain compared to the adult human and animal brain.[14] GABA receptor activity is regulated by subunit expression and composition. In the neonatal brain, the α_4 and α_2 subunits are overexpressed and the α_1 subunit is under expressed compared to adult brain.[21] Overexpression of the α_4 subunit decreases GABA receptor sensitivity to benzodiazepines.[21] The two classes of GABA receptors, $GABA_A$ and $GABA_B$, have distinct functions. In the first postnatal week, activation of $GABA_A$ receptors causes membrane depolarization rather than the hyperpolarization typical of

mature GABA-ergic synapses.[14] This depolarization is due to a reversed chloride gradient in the developing brain attributable to decreased expression of the chloride exporter potassium-chloride co-transporter (KCC2) and the overexpression of the calcium importer, NKCC1.[22] Full maturation of these receptors is reached within 2 years of age.

Clinical Course

Neonatal seizures can significantly affect mortality and morbidity. Current therapies have improved the outcomes for neonatal seizures; however, long-term neurological sequelae have not changed. The Collaborative Perinatal Project, which studied the outcomes of neonatal seizures in 277 infants from 1959-1966, reported a 35% mortality rate with 30% of the survivors having long-term adverse neurological outcomes at 7 years of age including cerebral palsy (13%), intelligence quotient less than 70 (19%), and epilepsy (20%).[23] In a recent study, mortality was found to be much lower at 7%, but poor long-term neurological outcomes remained the same as decades earlier.[24]

Long-term neurological outcomes can include cognitive and behavioral disabilities and epilepsy. The severity of adverse outcomes can be associated with severe encephalopathy, cerebral dysgenesis, complicated intraventricular hemorrhage, infections in the preterm infants, abnormal neonatal EEGs, and the need for multiple drugs to treat the neonatal seizures.[25] Cognitive changes can consist of mental retardation and learning disabilities. Incidence of cognitive effects has been reported to be 20 to 67%.[25,26] These changes are thought to be due to loss of hippocampal synchronized network discharge or

loss of hippocampal pyramidal cells.[27] Neonatal seizures may have subtle neurodevelopmental vulnerability on behavioral and learning abilities. This may become evident in specific learning difficulties or poor social adjustment. In a study of survivors of neonatal seizures, mortality outcome of the children was low. However, the entire sample displayed abnormal neuropsychological development in terms of intelligence test profile and subtest scatter, or development of spelling, or development of memory.[28]

Epilepsy is a common long-term neurological outcome associated with neonatal seizures. For many children, permanent fixed brain injuries, such as resolving stroke, ischemia, or traumatic lesions, serve as the nidus for future epilepsy.[11] Repeated neonatal seizures may have "instructed" the brain how to have future seizures, resulting in a persistent lowering of the seizure threshold and the development of chronic epilepsy. Approximately 17 to 30% of survivors of neonatal seizures experienced one or more seizures up to 7 years of age, nearly two-thirds of the seizures occurred within the first 6 months of life.[11,26,28,29] Little research has been done to examine development beyond seven years of age.

1.4.2. Infancy

Infantile Spasms

Infantile spasms are a unique disorder that occurs almost exclusively in infancy (the first year of life). Infantile spasms usually are associated with developmental retardation or deterioration and a characteristic EEG pattern that together form a syndrome.[30]

Epidemiology and Etiology

The onset of infantile spasms most commonly occurs between 3 and 8 months of age with a peak at 6 months.[2,30] Over 90% of cases begin before 12 months of life.[4] It is slightly more common in males, accounting for about 60% of cases. The incidence of infantile spasm is 1 per 2,000-4,000 live births and the prevalence rate is 0.15-0.2 per 1,000 children of age 10 years or younger.[4] The information for familial occurrence is limited. The percentage of patients who have a family member with infantile spasms has ranged from none to 7%.[2,4]

Infantile spasms have multiple causes, but the mechanisms by which they occur are not completely understood. Infantile spasms are classified into three main categories – genetic, structural/metabolic, and unknown according to the ILAE .[4,31] Cryptogenic infantile spasms is the terminology used when there are no apparent causes identified although a cause is suspected, usually because the child is developmentally delayed or has some other neurological impairment before the onset of the spasms. Cryptogenic cases account for 9%-15% of infantile spasms cases.[4] Idiopathic is used to describe children with no identifiable cause and who have a normal neurological examination and normal development prior to the onset of infantile spasms. The number of genes associated with infantile spasms are rapidly increasing (ARX, CDKL5, FOXG, GRIN1, GRIN 2A, MAGI, MEF2C, SLC25A22, SPTANI, STXBP1, and 15q11q13).[31] Symptomatic cases are those where there is structural brain abnormality or metabolic cause.

There are various underlying disorders for symptomatic cases that can be classified into prenatal, perinatal, and postnatal groups. Accounting for over 40% of total cases, prenatal etiologies include CNS malformations, chromosomal abnormalities, congenital central nervous system infections, and rarely, in-born error of metabolism. Perinatal precipitants include HIE and hypoglycemia.[30] Postnatal factors include intracranial infections, hypoxic-ischemic insults, and brain tumors. Tuberous sclerosis is a major cause of infantile spasms, with up to 50% of all patients with tuberous sclerosis presenting with infantile spasms which peak between 4 and 6 months of age.[32]

Clinical Course

Medical treatment for infantile spasms continues to be a challenge and there is no conclusive evidence that treatment alters the developmental or mental outcome in these patients. The prognosis of infantile spasms in terms of neurodevelopmental progress and the development of other types of seizures remain poor despite treatment. The clinical spasms and EEG hypsarrhythimia (abnormal interictal pattern consisting of high amplitude and irregular waves and spikes) pattern tends to disappear spontaneously by 3-4 years of age. However, up to 60% of these children will go on to develop other types of seizures.[4]

Reviews of studies of long-term outcomes for patients with infantile spasms suggest only 16% of the patients had normal development.[33] Mental retardation is common in patients. Symptomatic patients experience a higher rate of seizure occurrence (54%) compared to cryptogenic patients (23%).[4,33] In addition, the percentage of cryptogenic

patients with normal development (51%) was significantly higher than that of symptomatic patients (6%) particularly if treatment has been started within 1 month.[31,33] It is suggested that better initial control of spasms by hormonal treatment in cryptogenic or idiopathic cases of infantile spasms may lead to improved developmental outcome. There is growing evidence that longer duration of spasms is associated with poorer neurodevelopmental outcomes such as developmental delay and autistic behavior.[34] Spontaneous remission of spasms and disappearance of hypsarrhythimia in untreated patients have been reported in 25% of children by 1 year of age, but overall, the developmental outcome in infantile spasms is poor.[35]

Febrile Seizures

The ILAE defines a febrile seizure as "a seizure in association with a febrile illness in the absence of a CNS infection or acute electrolyte imbalance in children older than 1 month of age without prior afebrile seizures". The temperature associated with the febrile illness must be greater than 38.4°C.[2] Febrile seizures can be classified as either simple or complex. A simple febrile seizure is isolated, brief, and generalized. Conversely, a complex febrile seizure is focal, multiple, or prolonged, lasting more than 10 or 15 minutes.

Epidemiology and Etiology

Febrile seizures are the most common form of childhood seizures, affecting between 2% and 4% of children in the United States and Western Europe, 9% to 10% of children in Japan[36], and up to 14% of children in Guam.[37] The peak incidence of febrile seizures is

at approximately 18 months of age with most commonly occurring between 6 months and 5 years of age.[38]

The factors associated with increased risk of febrile seizures include: (1) a first or second degree relative with a history of febrile seizures, (2) a neonatal nursery stay of >30 days, (3) developmental delay, or (4) attendance at day care. There was a 28% chance of experiencing at least one febrile seizure for children with two of these factors.[39] Approximately one-third of children with a first febrile seizure will experience a recurrence; 10% will have three or more febrile seizures.[38] The most consistent risk factors for recurrent febrile seizures are family history, onset of first febrile seizure at <18 months of age, peak temperature, and the duration of the fever prior to the seizures.[38]

Clinical Course

The morbidity and mortality associated with febrile seizures are extremely low. Multiple studies have demonstrated no evidence of permanent motor deficits following febrile seizures.[40-44] These studies have also shown that cognitive abilities and school performance of children with febrile seizures were similar to those of controls. Even prolonged febrile seizures do not appear to be associated with adverse cognitive outcomes.[43,45]

Myoclonic Epilepsies in Infancy and Early Childhood

There are three types of myoclonic epilepsies in infancy: benign, severe, and myoclonic-astatic. The features of the main types of myoclonic epilepsies occurring in younger children are shown in Table 2. This review will focus on Dravet syndrome.

Table 2: Distinctive Features of the Main Types of Myoclonic Epilepsies[2]

Syndrome	Age at Onset	Seizure Type	Associated Conditions/Etiology	Prognosis/Outcome
Benign myoclonic epilepsy in infancy	4 m-3 y	Bilateral jerks; spontaneous or reflex	Idiopathic	Excellent in most; self-limited condition
Severe myoclonic epilepsy (Dravet syndrome)	3 m-2 y	Febrile, convulsive, unilateral, sleep-related, falsely generalized; myoclonic, atypical absence	Progressive mental decline	Poor to very severe; mental handicap; high risk of SUDEP
Myoclonic-astatic epilepsy (Doose syndrome)	1-4 y	Myoclonic; astatic; myoclonic-astatic; absence status	Idiopathic; progressive mental deterioration in some; ion channel disorder	Excellent to poor; self-limited in some with offset in childhood

Epidemiology and Etiology

The frequency in the general population is not well known, but has been estimated at 1 per 40,000 births.[46] Dravet syndrome represents 3% to 5% of epilepsies with onset in the first year of life.[2,47] Males are more often affected, with a sex ratio of 2.[46,47]

A family history of febrile seizures occurs in up to 71% of Dravet syndrome cases.[48] In addition, mutations in the sodium-channel gene SCN1A have been found in all seven

probands. Subsequent studies have confirmed the presence of this mutation in most, but not all patients, with a lesser prevalence in border-line or atypical cases.[49] Approximately 70% of patients are mutationally positive.[50]

Clinical Course

The prognosis for patients with Dravet syndrome is poor. Seizures tend to persist, all patients have cognitive dysfunction, and mortality is high, especially when patients reach adolescence and adulthood.[46] The mortality rate is approximately 15.9-18%.[49] After the regression between ages 1-5 years, mental impairment remains more or less stable. Slowness, perseverations, and language impairment are prominent; motor difficulties and orthopedic problems are also common.[4]

1.4.3. Childhood

Lennox-Gaustat Syndrome

Lennox-Gaustat syndrome (LGS) is a pediatric epilepsy syndrome characterized by multiple seizure types; mental retardation or regression; and abnormal findings on electroencephalography.

Epidemiology and Etiology

LGS accounts for 1%-4% of all cases of childhood epilepsy, but 10% of cases start in the first 5 years of life.[2,4] The annual incidence of LGS in childhood is 2 per 100,000 children while its prevalence ranges from 0.1 to 0.28 per 1,000 in Europe and the United States. The mean age at epilepsy onset is 26 to 28 months (range 1 day-14 years).[51,52]

LGS can be classified according to its suspected etiology as either idiopathic or symptomatic. Patients may be considered to have idiopathic LGS if there is normal psychomotor development before the onset of symptoms, if there are no underlying disorders or definite presumptive cause, and if there are no neurologic or neuroradiologic abnormalities. In contrast, patients have symptomatic LGS if there is an identifiable cause for the syndrome. Pathologies responsible for LGS include encephalitis/meningitis, tuberous sclerosis, brain malformations, birth injury, hypoxic-ischemic injury, frontal lobe lesions, and trauma. Infantile spasms precede the development of LGS in 9% to 41% of cases.[2,4] Population-based studies have found that 22% to 30% of patients have idiopathic LGS, and 70% to 78% have symptomatic LGS.[51,53,54]

Clinical Course

The long-term prognosis for patients with LGS is poor. Several studies have prospectively followed cohorts of children with LGS and demonstrated persistence of typical characteristics such as frequent seizures and different seizure types over time in many of these children. Mortality is reported to range from 3% (mean follow-up of 8.5 years) to 7% (mean follow-up of 9.7 years).[54]

Cognitive dysfunction, psychiatric symptoms, and multiple seizure types are common in patients with LGS. Factors associated with cognitive dysfunction are symptomatic LGS, a previous history of infantile spasms, onset of symptoms before 12 to 24 months of age,

and higher seizure frequency. A significant correlation exists between age of onset of seizures and mental deterioration, with a favorable cognitive outcome more likely to occur in patients with a later age of LGS onset.[55]

Psychiatric symptoms in young children consist of mood instability and personality disturbances, while slowing or arrest of psychomotor development and educational progress characterize the neuropsychological symptoms. Character problems predominate in older children, and acute psychotic episodes or chronic forms of psychosis with aggressiveness, irritability, or social isolation may occur.[54]

Several types of seizures occur in LGS including tonic, atonic, myoclonic, and atypical absences, often associated with other less common types.

Absence Epilepsies

Childhood absence epilepsy (CAE) is characterized by a brief, sudden lapse of consciousness that occurs in school age children. Based on earlier studies, CAE has been considered a benign disorder with relatively easily attained seizure control and minimal involvement of cognition and behavior.[2]

Epidemiology and Etiology

Absence seizures comprise 2% to 11% of seizure types in all ages. Childhood absence epilepsy accounts for 8% to 15% of all childhood epilepsies with an incidence of 4.7-8.0 per 100,000 children between the ages of 1 and 15 years.[56,57] The average age at

presentation is 6 years (range 2-10 years) with previous febrile seizures reported in 11%.[58]

The etiology of absence epilepsy is suggested to be complex involving both acquired and inherited factors. Recent investigations found calcium channels and GABA receptors to be associated with absence epilepsy.[2,4]

Clinical Course

Children with absence epilepsy have a good prognosis. Most (65%) become and remain seizure free with the average age of cessation of absence seizures being 10.5 years.[57] A small population (7%-9%) have persistent absence seizures, 13%-30% develop GTCS and 15% develop myoclonic seizures.[2,4] Children with CAE are in the normal cognitive range, but when compared to matched controls without epilepsy, have significantly lower full IQ scores.[59] In addition, they are more likely to have linguistic difficulties (43%) and a psychiatric diagnosis (61%), particularly attention-deficit/hyperactivity disorder (ADHD) and anxiety disorders compared to controls.[59]

Benign Epilepsies

Benign epilepsy is the most common type of seizures in children. They are characterized by epileptic seizures that are easily treated, or require no treatment, and remit without sequelae.[3] There are a number of syndromes that fall into this category: benign familiar neonatal seizures, benign infantile spasms, Panayiotopoulos syndrome (PS), benign childhood epilepsy with centrotemporal spikes (BECTS), and benign childhood occipital

epilepsy. Table 3 summarizes the main features of the three idiopathic childhood focal epilepsies.

Epidemiology

Onset of BECTS is between 1-14 years, in 75% of patients, onset is between 7 and 10 years, and there is a peak at 8-9 years.[60] There is a male predominance of 1.5 times male compared to female. Prevalence is around 15% in children with seizures aged 1-15 years. Incidence is 10-20 per 100,000 children aged 0-15 years.[61] Age at onset for PS is 1-14 years with a peak at 4-5 years. In 75% of cases, onset occurs at 3-6 years of age. Prevalence is around 13% in children 3-6 years old with one or more non-febrile seizures and 6% in the 1-15 years old in those children with epilepsy.[2,60] In the general population, 2-3 per 1,000 children may be affected. Childhood occipital epilepsy (Gastaut type) is a rare condition with a prevalence of 0.2%-0.9% of all epilepsies, and 2%-7% of benign childhood epilepsies.[4,60] It is estimated to account for 0.15% of all focal epilepsies in childhood. Age at onset is 3-15 years with a mean around 8 years of age.[4,60]

The benign epilepsies have an unknown etiology, but suggested to be most likely genetic. Linkage to chromosome 15q14 has been suggested for BECTS, but not confirmed in several studies.[2,4,60,62] Prevalence of febrile seizures is common in all benign epilepsies: 10-25% in BECTS, 16-45% in PS, and 14% in childhood occipital epilepsy.[2,4,60,61]

Table 3: Comparison of Clinical Features of Three Idiopathic Childhood Focal Epilepsies

	Panayiotopoulos Syndrome	BECTS (Rolandic Epilepsy)	Childhood Occipital Epilepsy (Late-Onset, Gastaut Type)
Prevalence among children 1-15 years with febrile seizures, %	6	15	1-2
Mean age at onset (range), years	4-5 (1-14)	8-9 (1-15)	8-9 (3-16)
Sex prevalence, %	54 males	60 males	50 males
Prognosis	Excellent	Excellent	Uncertain

Clinical Course

Long-term prognosis is good in BECTS and PS with remission in over 90% of cases by age 12 for BECTS and 1-2 years after seizure onset for PS.[2,4,60] Antiepileptic drug (AED) therapy is often not needed in these syndromes as one-third of patients have a single seizure and only 5-10% will have more than 10 in the case of PS. The majority of patients have less than 10 seizures; 10-20% have a single seizure and 10-20% have frequent seizures, but these also remit with age.[60] The prognosis for childhood occipital epilepsy is variable. A majority (50-60%) of patients achieve remission within 2-4 years from onset.[2,4,60] Seizures show a fairly good response to AEDs in more than 90% of patients.[2,4,60]

1.4.4. Adolescence

Juvenile Myoclonic Epilepsy

Idiopathic generalized epilepsies (IGE) are a distinct group of epilepsy syndromes. Syndromes of idiopathic generalized epilepsies are differentiated on the basis of the

predominant seizure pattern and age of onset. Juvenile myoclonic epilepsy (JME) is a common epilepsy syndrome characterized by myoclonic seizures with or without other seizure types that occurs in adolescence. It represents approximately 10% of all epilepsies.

Epidemiology and Etiology

JME age of onset ranges from 8-36 years, with a peak onset between 12 and 18 years.[63,64] The incidence of JME is estimated to be 1 per 100,000 persons, with a prevalence of 0.1-0.2 per 100,000. The frequency of JME has been reported to be 5-10% of all epilepsies and 18-40% of IGE.[63,64]

A number of genetic abnormalities have been implicated for JME. Five Mendelian JME genes have been identified, namely, *CACNB4* , *CASR* , *GABRa1* , *GABRD*, and *Myoclonin1/EFHC1*.[65] A few mutations, including the KCNQ potassium channels and the ClC-2 chloride channel have been suggested.[66] In addition, three SNP alleles in BRD2, Cx-36, and ME2 and microdeletions in 15q13.3, 15q11.2, and 16p13.11 also contribute risk to JME.[65] The inheritance of IGEs and specifically JME is complex as it does not follow a well-defined Mendelian pattern.

Clinical Course

The most unique feature of JME among IGEs is that it is not outgrown and requires lifelong treatment, as the rate of recurrence is very high after discontinuation of medication, even after a long remission.[67] However, population-based studies have

reported medical and social outcomes of JME patients with a 20-65 years range of follow-up and suggest there is a possibility to become seizure free.[68-70] In one study 54.5% of patients became seizure-free and 22.2% discontinued AED treatment and 39% eventually stopped AEDs and became seizure free in another study.[69,70]

1.5. SUMMARY

This chapter gives a broad overview of childhood epilepsies. It is estimated that the prevalence of lifetime epilepsy/seizure disorder for the US child population is 1% which is slightly higher than prior estimates.[2] Further study is warranted for pathophysiologic processes contributing to the development of comorbid conditions and may provide clues to the etiology of seizures disorders. This will advance the development of therapies that might be useful in these specific epilepsy syndromes.

CHAPTER 2: IMPACT OF DEVELOPMENT ON PHARMACOKINETICS AND PHARMACOTHERAPY IN CHILDHOOD EPILEPSY: EMPHASIS ON TOPIRAMATE

2.1 DEVELOPMENTAL PHARMACOKINETICS

Pharmacotherapy for children is challenging because there is a lack of prescribing information. Pathophysiologic differences between children and adults result in age-related differences in PK and drug effect. Changes in development and how this may affect PK properties of absorption, distribution, metabolism and excretion are summarized below.

2.1.1 Absorption

The rate and extent of gastrointestinal absorption is primarily dependent upon pH dependent passive diffusion and motility of the stomach and small intestine in addition to gastric contents, gastric emptying time, and absorbing surface.[71-73] The pH of the stomach is between 6 and 8 at birth, decreases to around 2-3 within 48 hours after birth, returns to neutral over the next 24 hours, and remains that way for the next 10 days.[71,74] Then, it slowly declines again until it reaches adult values at about 20-30 months of age with the lower limit of adult values are achieved by approximately 3 months of age.[72,75]

Gastric emptying is prolonged and erratic; it approaches adult values within the first 6-8 months of life.[73,76] Gastric emptying is prolonged in neonates following reduced motility and peristalsis. In infants and children, intestinal transit time is reduced as a result of increased intestinal motility.[76,77] In both neonates and infants, additional factors such as the relative immaturity of the intestinal mucosa, immature biliary function, high levels of β-glucuronidase activity and variable microbial colonization may also affect absorption.[71,72]

Rectal absorption is generally higher in newborns and infants consequent to efficient translocation across the rectal mucosa, but also reduced presystemic drug clearance due to immaturity of many drug metabolizing enzymes in the liver.[71,72] In contrast to rectal absorption, intramuscular administration is erratic in the neonate given that the muscular blood flow is low, muscular contractions are irregular and there is an increased percentage of water per unit of muscle mass.[71,72] In contrast, the intramuscular route is very efficient in infants given a relatively high blood flow and more muscular contraction.[71,72] Developmental differences in drug absorption between neonates, infants and older children are summarized in Table 4.

Table 4: Characteristics of Drug Absorption Physiology During Development

	Newborns	Infants	Children
Gastric emptying time	Irregular	Increased	Slightly increased
Gastric pH	>4	4 – 2	Normal (2-3)
Intestinal motility	Reduced	Increased	Slightly increased
Absorbing surface	Reduced	Normal	Adult pattern
Mucous thickness	Increased	--	Adult pattern
Microbial flora	Colonization	Adult pattern	Adult pattern
Biliary function	Not fully developed	Developed	Adult pattern
Muscular blood flow	Reduced	Increased	Adult pattern
Skin permeability	Increased	Increases	Near adult pattern

Direction of alteration given relative to expected normal adult pattern.
Adapted from Morselli, 1983 and Kearns, 2008.

2.1.2 Distribution

During development, changes in body composition occur that can affect drug distribution. These changes include total body water (TBW), extracellular water (ECW), and body fat. The most dynamic changes occur in the first year of life with the exception

of body fat, which is reduced by approximately 50% between 10 and 20 years of life.[78] In general, TBW and extracellular water decrease with age and body fat increases with age. The magnitude of the possible difference between a newborn and a child or an adult regarding the volume of distribution will depend on the physicochemical properties of the drug and on the maturational and physiopathological status of each patient.[72]

Plasma protein binding is significantly reduced in newborns given these factors: reduced plasma albumin concentration associated with the presence of fetal albumin, lower concentration of plasma globulin, high plasma concentration of unconjugated bilirubin associated with an elevated plasma concentration of free fatty acids, and a relatively acidic blood pH.[72] In infants, plasma protein binding of drugs continues to be reduced because of the lower albumin and globulin contents. The amount of reduction is variable and in most cases values comparable to adult values are reached at 10-15 years of age.[72] Developmental differences in drug distribution between neonates, infants and older children are summarized in Table 5.

Table 5: Characteristics of Disposition Physiology During Development

	Newborns	**Infants**	**Children**
Plasma albumin	Reduced	Near normal	Near adult pattern
Fetal albumin	Present	Absent	Absent
Total proteins	Reduced	Decreased	Near adult pattern
Globulin	Reduced	Decreased	Near adult pattern
Serum bilirubin	Increased	Normal	Adult pattern
Free fatty acids	Increased	Normal	Adult pattern
Blood pH	<7.4	~7.4	7.4
Adipose tissue	Scarce	Reduced	----
Extracellular water	Increased	Increased	Adult pattern
Total body water	Increased	Increased	Adult pattern

Direction of alteration given relative to expected normal adult pattern.
Adapted from Morselli, 1983 and Kearns, 2008.

2.1.3 Metabolism and Excretion

The majority of elimination of drugs occurs through either renal or biliary excretion of the unchanged parent drug, hepatic biotransformation to metabolites, or a combination of both. Age-related changes in drug metabolism and excretion are summarized in Table 6 and Table 7. At birth, the glomerular filtration rate (GFR) is approximately 40 mL/min/1.73 m^2 in the full-term neonate. GFR increases steadily to 50% to 75% of adult function by 6 months.[72,76] Tubular secretion matures by 7 months to a year. In addition, there is a glomerular/tubular imbalance, due to the more advance maturation stage of glomerular function. Such an imbalance may persist up to 6 months of age.[72] Full maturation of renal function is attained by 1 year of age. Knowledge of transporter proteins that participate in active renal excretion and reabsorption is limited.[76] Data on biliary excretion during development has not been studied. However, it is likely to be reduced in the early weeks of life.

Table 6: Characteristics of Renal Excretion Physiology During Development

	Newborns	Infants	Children
Glomerular filtration	Reduced	Normal	Normal adult values
Tubular secretion	Reduced	Near normal	Normal
Tubular resorption	Reduced	Near normal	Normal
Glomerular/tubular imbalance	Present	Present (6 months)	Normal
Proteins in filtrate	Present (30%)	Low to absent	Normally absent
Urinary pH	Low	~Low	Normal
Kidney weight/body weight ratio	Increased	Increased	Near adult values

Direction of alteration given relative to expected normal adult pattern.
Adapted from Morselli, 1983 and Kearns, 2008.

In general, most of the enzymatic activities responsible for metabolic degradation of drugs are reduced in the neonate.[72] Phase I reactions mainly involve oxidation, reduction, and hydrolysis. The primary phase I enzymes of metabolism include the cytochrome P450 (CYP) enzymes. The developmental patterns of the ontogeny of a number of important drug metabolizing enzymes have been described.[72] For example, CYP2D6 is low to absent in the fetal liver but present at 1 week of age. It has poor activity (20% of adult) by 1 month reaching adult levels by 12 months of age. CYP2C19 and CYP2C9 are absent from the fetal liver. There is low activity in the first 2-4 weeks of life with adult activity reached by approximately 6 months of age. Activity may exceed adult levels during childhood and declines to adult levels after the conclusion of puberty. CYP1A2 is not present in appreciable levels in human fetal liver.[76] Adult levels are reached by approximately 4 months and exceeded in children at 1–2 years of age.[76] Once again, adult activity is reached after puberty. CYP3A7 is the fetal form of CYP3A4 which is functionally active (and inducible) during gestation.[76] It disappears by 1–4 weeks of postnatal when CYP3A4 activity predominates, but remains present in approximately 5% of individuals. CYP3A4 has extremely low activity at birth reaching approximately 30–40% of adult activity by 1 month and full adult activity by 6 months. The activity may exceed adult activity between 1–4 years of age, decreasing to adult levels after puberty.[76]

Phase II biotransformation reactions generally are conjugation reactions catalyzed by transferases. The major phase II enzymes are uridine diphosphate-glucuronosyltransferases, sulfotransferases, N-acetyltransferases, glutathione S-transferases and methyltransferases. The ontogeny of UGT is isoform specific. In

general, adult activity is reached by 6-24 months of age.[71,76] Sulfotransferases is also isoform specific and appears to develop more rapidly than uridine diphosphate-glucuronosyltransferases. Activity for some isoforms may exceed adult levels during infancy and early childhood.[71,76]

Table 7: Characteristics of Metabolism Physiology During Development

	Newborns	Infants	Children
Hepatic microsomal activities	Reduced	Increased	Slightly increased
Blood esterase activity	Reduced	Normal (by 12 months)	Adult pattern
UGT activity	Reduced	Increased	Near adult pattern
Protein gamma	Absent	Present	Present
Liver blood flow	Reduced	Increased	Near adult pattern
Synthetic reactions	Reduced	Increased	----
Liver weight/body weight ratio	Increased	Increased	Slightly increased

Direction of alteration given relative to expected normal adult pattern.
Adapted from Morselli, 1983 and Kearns, 2008.

2.2 PHARMACOTHERAPY

Roughly 17 drugs are used to treat childhood epilepsies. As topiramate is the focus of this dissertation, this section begins with a review of its PK, pharmacology, and use in clinical epilepsy. Thereafter, all other drugs used as oral maintenance therapy are discussed.

2.2.1 Topiramate

TPM is a second-generation antiepileptic medication approved in 1996 for seizures. It was later approved for migraine prophylaxis, and in 2012 for weight loss in combination with phentermine.

Physical-Chemical Properties

TPM is 2,3:4,5-Di-O-isopropylidene-ß-D-fructopyranose sulfamate (Figure 3). The molecular formula is $C_{12}H_{21}NO_8S$ and its molecular weight is 339.36. TPM is a white crystalline powder with a bitter taste.[79] It is most soluble in alkaline solutions containing sodium hydroxide or sodium phosphate and having a pH of 9 to 10 and is freely soluble in acetone, chloroform, dimethylsulfoxide, and ethanol.[79] Its solubility in water is 9.8 mg/mL.[79] Its saturated solution has a pH of 6.3.

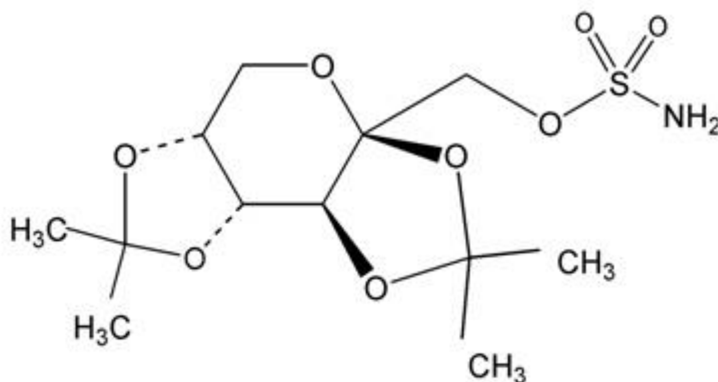


Figure 3: Chemical Structure of Topiramate

Indications

TPM is approved for monotherapy in patients greater than 2 years of age with partial onset or primary generalized tonic-clonic seizures.[79] It is also approved for adjunctive therapy for adults and pediatric patients (2-16 years of age) with partial onset seizures or primary generalized tonic-clonic seizures, and in patients greater than 2 years of age with seizures associated with Lennox-Gastaut Syndrome.[79] TPM is approved for treatment for adults and adolescents 12 years or age and older for prophylaxis of migraine headaches.[79]

Pharmacology

TPM has a broad spectrum of action, although the precise mechanisms for its anticonvulsant and migraine prophylaxis effects are unknown. Preclinical studies have revealed four properties that may contribute to TPM's efficacy for epilepsy and migraine prophylaxis. At pharmacologically relevant concentrations, TPM blocks voltage-dependent sodium channels, augments the activity of the neurotransmitter GABA at some subtypes of the $GABA_A$ receptor, antagonizes the AMPA and kainite subtype of glutamate receptor, and inhibits the carbonic anhydrase enzyme, specifically isozymes II and IV.[80]

Neuroprotection

Studies have demonstrated that TPM exhibits neuroprotective properties. Neuroprotection has been reported for topiramate in models of status epilepticus and cerebral ischemia (Summary in Table 8).[81-88] A recent study has shown diminished apoptosis in the CA1 region and dentate gyrus of hippocampus using a 80 mg/kg/day dose of TPM.[82] In another study, there was a reduction of neuronal cell loss in the high dose group (loading dose 50 mg/kg; maintenance dose 20 mg/kg/day).[86] Liu et al studied the combination of memantine and topiramate for hypoxic-ischemia brain injury. They found no elevated apoptosis (programmed cell death) in the cortex, hippocampus, thalamus, and striatum using a combination of memantine (loading dose 20 mg/kg; maintenance dose 1 mg/kg BID) and TPM (loading dose 40 mg/kg; maintenance dose 1 mg/kg BID). Follett et al. studied the protective effect of intraperitoneal doses of 10, 30, and 50 mg/kg of topiramate in a rat model of periventricular leukomalacia, or white

matter injury.[81] Only the 30 mg/kg topiramate dose significantly attenuated lesion severity and motor deficits.[81] In several models, topiramate has a dose-dependent effect on reducing neuronal damage when administered after injury.

Table 8: Topiramate Neuroprotective Studies

Study	Injury	Animal	Dose (mg/kg)	Result
Kurul et al.[82]	Hyperoxia-induced neurodegeneration	Rat pups	80 mg/kg/day	Diminished apoptosis in CA1 region and dentate gyrus of hippocampus
Liu et at.[88]	Hypoxic-ischemia	Rat pups	Loading 40 mg/kg; maintenance 1 mg/kg	Reduction of neuronal cell less in combination therapy
Schubert et al.[86]	Perinatal hypoxia-ischemia	Piglets	10 and 20 mg/kg/day	Reduction of neuronal cell loss in high dose animals
Follett et al.[81]	Periventricular leukomalacia	Rat pups	10, 30, or 50	30 mg/kg has protective effect on lesions
Koh et al.[89]	Hypoxic induced seizures	Rat pups	30 & NBQX	Repeated dose prevents susceptibility to hippocampal injury
Cha et al.[87]	Status epilepticus	Rats	80 mg/kg/day	After 4 weeks of treatment no different in cell loss
Lee et al.[83]	Global ischemia	Gerbils	50, 100, or 200	100 and 200 mg/kg reduced neuronal damage
Niebauer et al.[84]	Status epilepticus	Rats	20, 40, or 80	Reduced degeneration at all doses
Yang et al.[85]	Cerebral ischemia	Rats	20 or 40	Reduced infarct volume and deficit scores, dose dependent effect

Pharmacokinetics

Absorption

Absorption of TPM is rapid with peak plasma concentrations occurring 2 hours after oral administration. Absorption is linear with dose.[80] Administration of TPM with food decreases the rate of absorption but not the extent. Food decreases the mean maximum concentration by approximately 10% and delays the mean time to maximum concentration by approximately 2 hours.[90] Initial studies of investigational IV TPM in twenty adult patients and twelve healthy volunteers on oral TPM estimated the absolute bioavailability of TPM to be close to 100%.[91,92]

Distribution

Topiramate is 9-17% bound to plasma proteins, but exhibits saturable binding to carbonic anhydrase in the red blood cells (RBCs).[93] The apparent volume of distribution is 0.6-0.8 L/kg, which is similar to the distribution of total body water.[80]

Metabolism and Excretion

Twenty to 30% of topiramate is metabolized when administered in the absence of enzyme inducers.[94] The exact enzymes involved in the biotransformation of TPM are unknown, but it appears to undergo CYP-mediated oxidation.[95] Multiple metabolites are formed by hydroxylation, hydrolysis, and glucuronidation.[96] Doose and Streeter state that the CL/F of topiramate was reduced by 22 % in “hepatically impaired patients” compared with healthy subjects with epilepsy.[97] When topiramate is administered with an enzyme

inducer, up to 50% of the dose undergoes metabolism.[80,98,99] Topiramate inhibits CYP2C19.[96]

Approximately 70% of a topiramate dose is excreted unchanged in the urine. The clearance of TPM is reduced significantly in patients with renal dysfunction. In a study investigating the effect of renal dysfunction on TPM clearance, the clearance was reduced by 42% in patients with a creatinine clearance 30-69 ml/min and 54% in patients with a creatinine clearance less than 30 ml/min.[100] Oral TPM clearance (CL/F) is approximately 1 L/hr to 2 L/hr when given alone.[90,101-103] Studies have found clearance to be substantially higher and half-life to be lower in children than in adults.[104-106]

TPM PK in children and adolescents was determined from a single-center, open-labeled outpatient trial of 18 patients.[107,108] In patients 4-17 years old, clearance values were 54% greater in children when TPM was administered in the presence of enzyme-inducing drugs and 44% greater in the absence of enzyme-inducing drugs. There are a limited number of reports describing the PK in infants under 2 years of age. Studies in younger children (6 months-4 years) have found a plasma clearance slightly higher than that reported for children and adolescents. Again the plasma clearance was higher in infants on concomitant enzyme-inducing AEDs than in those on non-enzyme-inducing AEDs.[104,105] Mikaeloff et al studied the PK of TPM in 9 children aged 6 months to 2 years with epilepsy. This study showed the apparent oral clearance of TPM was higher in infants taking enzyme inducing AEDs (85.4 $\pm$ 34.0 ml/h/kg) compared with those taking neutral AEDs (46.5 $\pm$ 12.8 ml/h/kg).[104] Similar studies have found clearance to be

substantially higher in infants (age 24-29 months) than in adults.[104,105] Two small (N = 40) studies have investigated oral TPM PK and safety in neonates with HIE.[109,110] These studies examined the effect of hypothermia on oral TPM plasma concentrations over a 72 hour period. Babies were assigned to one of two core temperature (31°C vs. 33°C) protocols. Greater than 50% of the patients also received phenobarbital. These studies found the range of plasma TPM concentration to be between 1.5-20 mg/L for mild hypothermia and 7-30 mg/L for deep hypothermia.

Half-Life

When TPM is administered alone, the half-life is reported to be 30-80 hours.[90,92,102,111,112] When administered with enzyme inducers, the half-life decreases to 12-15 hours.[80,113,114] In children, studies have found half-life to be faster in infants than in adults (5-17 hours).[104-106] A summary of reported PK parameters for different age groups is presented in Table 9.[104-106,108,109,115,116]

Table 9: PK Parameters as a Function of Age

Age Group	Clearance (mL/hr/kg)	Volume of Distribution (L/kg)	Half-Life (hr)
Neonates	13 - 18	NC	29 - 49
Infants	7 - 110	0.3 - 2.13	6 - 14
Children (< 10 years)	20 – 213	NC	5 - 17
Children (10 -17 years)	20 - 90	NC	5 - 17
Adults	14 – 30	0.6 - 0.8	30 - 80

NC: not calculated

Drug Interactions

The potential for drug interactions is a concern when administering multiple medications. Topiramate does not significantly alter the metabolism of other AEDs, although its metabolism can be changed by enzyme-inducing drugs. Several AEDs interfere with TPM metabolism through the CYP system. When co-administered with carbamazepine, phenytoin or valproic acid, topiramate concentrations were decreased by 40%, 48% and 17%, respectively.[98,117,118]

Adverse Effects

The most common adverse events associated with TPM are headache, dizziness, fatigue, somnolence, anorexia, insomnia, and hyperesthesia. Adverse events that are likely to have been related to the carbonic anhydrase activity of TPM (e.g. paresthesia, changes in serum bicarbonate, kidney stones) are frequent (13-35%) but are not usually considered clinical relevant. The most frequent adverse events during maintenance therapy were headache (20%), reduced appetite (11%), and weight loss (11%).[2]

Topiramate Use in Children

Seizure Disorders

<u>Monotherapy in Children with Partial-Onset and Generalized-Onset Seizures</u>

The conclusion that topiramate is effective as initial monotherapy in children 2 to <10 years of age with partial onset or primary generalized tonic-clonic seizures was based on a pharmacometric bridging approach using data from the controlled epilepsy trials described in the topiramate product label. This approach consisted of first showing a

similar exposure-response relationship between pediatric patients down to 2 years of age and adults when topiramate was given as adjunctive therapy.[79] Similarity of exposure-response was also demonstrated in pediatric patients ages 6 to <16 years and adults when topiramate was given as initial monotherapy. Specific dosing in children 2 to <10 years of age was derived from simulations utilizing plasma exposure ranges observed in pediatric and adult patients treated with topiramate initial monotherapy.[79]

Adjunctive Therapy in Children with Partial-Onset Seizures

The effectiveness of topiramate as an adjunctive treatment for primary generalized tonic-clonic seizures in patients 2 years old and older was established in a 20-week multicenter, randomized, double-blind, placebo-controlled trials, comparing a single dose of topiramate to placebo.[119] Patients, aged 3 to 59 years, were randomized to topiramate (n=39) 175 mg, 225 mg, or 400 mg, or placebo (n=41). Primary generalized tonic-clonic seizures decreased by 56.7% in the topiramate group versus the placebo group (p=0.019). A seizure reduction rate of at least 50% was experienced by 56% of the topiramate patients versus 20% of the placebo group (p=0.001). For all generalized seizures, topiramate decreased the average monthly seizure rate by 42.1% versus 0.9% in the placebo group (p=0.003).

Adjunctive Therapy in Patient with Lennox-Gastaut Syndrome

The effectiveness of topiramate as an adjunctive treatment for seizures associated with Lennox-Gastaut syndrome was established in a multicenter, randomized, double-blind, placebo-controlled trial comparing a single dosage of topiramate with placebo in patients

2 years of age and older. Adjunctive topiramate therapy was effective in reducing the number of drop attacks or tonic-atonic seizures associated with LGS.[120] The median percentage reduction in drop attacks from baseline in the average monthly seizure rate was significantly greater in the topiramate group as compared to the placebo group (14.8% versus 5.1%, p=0.041). Thirty-three percent of subjects in the topiramate group had a 50% reduction in tonic-clonic seizures as well as drop attacks (p=0.002, compared to placebo).

Therapy in Patients with Infantile Spasms

TPM's efficacy against infantile spasms has been reported in at least 10 open-label trials.[2,121-130] Twenty patients with infantile spasms were treated with TPM monotherapy and 30% became spasm free.[125] In a prospective open-label study using TPM as initial therapy in 54 patients found that 57% of patients were seizure free for more than 24 months.[130] A prospective study in 40 children found 67.5% of all patients comprising 10 patients treated with TPM monotherapy and 17 with the combination of TPM and ACTH became seizure-free.[131] Greater than 75% reduction in the frequency of spasms was found in 4 of all patients and at least 50% reduction in 4 patients.[131] In the most recent study, fifty children with the mean age of 9.4±3.8 months were evaluated.[132] Cessation of all spasms occurred in 12 (48%) infants in TPM group and 4 (16%) in nitrazepam group. Eight (32%) children in the TPM group and 7 (28%) in the nitrazepam group had more than 50% reduction in spasms frequency.[132]

Therapy in Patients with Dravet Syndrome

Four open-labeled studies have examined TPM's efficacy for children with severe myoclonic epilepsy in infancy (Dravet Syndrome).[123,126,133,134] These studies showed that 17% of the patients became seizure free, and 56% had a reduction greater than 50% in seizure frequency (mean 11.2 months, with a range of 2-24 months). No patient experienced worsening of seizures with TPM therapy.[133] In a subset of a study, 3 of 5 Dravet patients responded to TPM therapy.[126] Additionally, in a prospective study of TPM 2 of 6 patients with Dravet's responded, but none became seizure-free.[123]

Adjunctive Therapy in Patients with Juvenile Myoclonic Epilepsy

TPM may have a role in treating JME although it seems to be more effective in controlling primary generalized tonic-clonic seizures and has low effect in controlling absences or myoclonic jerks.[119,135,136] A posthoc analysis of two multicenter, double-blind, randomized, placebo-controlled, trials of TPM for GTC seizures of nonfocal origin revealed that 22 patients with JME had been randomized to either TPM or placebo. A significantly higher number of patients in the TPM-treated arm experienced a reduction of 50% or greater in GTC seizures compared to the placebo arm.[135] The reductions of myoclonic and absence seizures were higher in the TPM arm, but the difference did not reach statistical significance.[135]

Adjunctive Therapy in Patients with Other Epilepsy Syndromes

There are several small open-labeled add-on studies suggesting that TPM is effective for absence epilepsy (N=5)[137], Doose syndrome (N=4)[126], and progressive myoclonic epilepsies (N=8)[138].

Previously Reported Population PK Models

Several population models for TPM have been reported in the literature. These models are displayed in Table 10. The populations explored in these models ranged from 6 months of age up to 85 years old. These models were both one- and two-compartment models and the covariates that were significant were age, enzyme inducing comedication, renal function, and weight.

Table 10: Topiramate Population Models in Literature

Study	Number of Subjects	Population	Model	Covariates	Conclusion
Vovk et al.[139]	26	Age: 8-54 years	One-compartment	CBZ dose and age	Clearance was 70% higher in patients co-treated with carbamazepine and was found to increase with patient age.
Jovanovic et al.[140]	78	Age: 19-67 years, Mono-or co-therapy	One-compartment with first-order absorption and elimination	CBZ dose, renal function	Individualization for patients on different CBZ dosing regimen
Bouillon-Pichault et al.[141]	22	Age: 6 months – 2 years,	One-compartment with first-order absorption and elimination with lag time	Enzyme inducers on clearance and effect of BW and age on volume	TPM dosage of 2-4 mg/kg/day provided the AUC reported in adults
Girgis et al[142]	1,217	Age: 2-85 years	Two-compartment with first-order absorption and elimination	Weight, age, and concomitant AEDs	No difference in PK-PD of TPM between adults and pediatric.
Ahmed et al.[143]	32	Age: 19-55 years, Healthy volunteers	Two-compartment with first-order absorption and elimination	Body weight	PD study

2.2.2 Other AEDs Useful in Treating Childhood Epilepsies

Adrenocorticotropin and Steroids

In the 1950s, the efficacy of adrenocorticotropin (ACTH) was first observed.[2,144] A number of studies have reported the efficacy of corticosteroids in infantile spasms and confirmed the utility of ACTH in the treatment of this disorder.[2] The epilepsy syndromes that respond uniquely to ACTH and corticosteroids therapy have an age-related onset during a critical period of brain development, as well as a characteristic regression or plateau of acquired milestones at seizure onset, and long-term cognitive impairment.

However, there are no clinical trials that compared the effectiveness of ACTH or oral corticosteroids versus placebo in controlling infantile spasms. Two randomized control trials revealed that ACTH was superior to prednisone in spasm cessation. When compared to vigabatrin, ACTH results in a higher and faster response rate in spasm control, with similar cognitive development after 9-44 months of treatment.[2,4] Thus, ACTH may be considered for initial monotherapy of infantile spasms, while oral corticosteroids may be considered as alternative first-line therapy.[2,144] In addition, a few studies have reported the use of ACTH for the treatment of myoclonic seizures and LGS with variable response rates.[145-147]

Mechanism of Action

The pathogenesis of infantile spasms, and therefore the mechanism of action of ACTH and steroids in this condition, is unknown, principally because there were no good animal models. However, a number of promising animal models have now been developed. The models currently used are either based on a specific cause of infantile spasms, such as loss of interneurons (aristaless-related homeobox ARX mouse), or propose a final common pathway underlying all causes of infantile spasms.[148] Recently developed models are the ARX-linked aristaless-related knockout and knock-in mouse model, the multiple-hit model, corticotrophin-releasing hormone model, the NMDA model and the tetradotoxin model.[148] No single model will replicate the human seizure exactly.

Various explanatory hypotheses have been formulated for the mechanism of action which have been discussed in detail by O'Regan and Brown.[147] These include the correction of deficient or dysfunctional enzymes or enzyme systems, changes in intracellular-extracellular electrolyte ratios, correction of hypoglycemia or low intracellular glucose, reduction in cerebral water content, an anti-inflammatory action, and immune modulation or suppression.

Adverse Effects

ACTH and steroids may cause significant side effects, including irritability, the development of Cushing syndrome, electrolyte disturbances (specifically hypokalemia), hyperglycemia, glucose intolerance, osteoporosis, infections, hypertension, and a usually reversible dilatation of the ventricular and extra-ventricular cerebrospinal fluid spaces.[149,150] However, the more serious side effects have been reported predominantly with ACTH rather than prednisolone and have included severe sepsis resulting in death[150,151] and cardiac hypertrophy.[152,153]

Finally, the long term use of steroids (over many months) to maintain any initial benefit or to try and modify any underlying and potentially evolving progressive disorder may be complicated by immunosuppression, glucose intolerance, and osteoporosis.[149]

Benzodiazepines

Benzodiazepines are a class of drugs that enhance the effect of the neurotransmitter GABA at the $GABA_A$ receptor, resulting in sedative, hypnotic, anxiolytic,

anticonvulsant, and muscle relaxant properties. Their high lipid solubility results in rapid central nervous system penetration and they are particularly useful as first-line agents in the management of status epilepticus and seizures. There are a number of benzodiazepines that include diazepam, lorazepam, midazolam, clonazepam, nitrazepam, clorazepate, and clobazam. The general mechanism of action and adverse effects are described here.

Mechanism of Action

Benzodiazepines augment inhibitory neurotransmission by enhancing the activity of GABA at the $GABA_A$ receptor.[2,144] When GABA binds to the $GABA_A$ receptor, it increases the opening of the chloride ion channel, which results in hyperpolarization of the membrane and reduction in neuronal firing.[2,144] Benzodiazepines do not activate the receptor directly but modulate GABA binding and enhance its effect by increasing the frequency of chloride ion channel opening. This increases the inhibitory tone at GABA synapses, which limits neuronal firing and results in reduced seizure activity.[2,144]

Adverse Effects

Respiratory and cardiovascular depression are the most common adverse effect of benzodiazepines.[2] Chronic treatment may be associated with sedation, fatigue, ataxia, cognitive dysfunction, drooling, and exacerbation of seizures.[2] Treatment should not be abruptly stopped as it may lead to withdrawal symptoms.

Long-term treatment of benzodiazepines can result in tolerance (when a subject's reaction to a specific drug and concentration of the drug is progressively reduced, requiring an increase in concentration to achieve the desired effect). Tolerance to one benzodiazepine does not necessarily result in tolerance to the others. The mechanism underlying tolerance is not clear but may involve down-regulation of $GABA_A$ receptors, altered postsynaptic sensitivity to GABA, or modification in the expression of genes that encode for the various $GABA_A$ receptor subunits.[154]

Carbamazepine

Carbamazepine (CBZ) is an iminodibenzyl derivative, structurally similar to the tricyclic antidepressants. CBZ is an effective treatment of epilepsy for partial and secondarily generalized seizures. It is extensively metabolized in the liver, with approximately 1% of the administered dose excreted in the unchanged form. The main oxidative pathway involves the formation of an active metabolite, carbamazepine- 10,11-epoxide, which possesses anticonvulsant properties similar to those of CBZ.[155]

Mechanism of Action

The main molecular mechanism of antiepileptic action of CBZ involves blocking voltage dependent sodium ion channels in cell membranes.[156] It stabilizes the inactivated state of voltage-gated sodium channels, making fewer of these channels available to subsequently open. This leaves the affected cells less excitable until the drug dissociates. CBZ has also been shown to potentiate GABA receptors made up of alpha1, beta2, and gamma2 subunits.[155]

Adverse Effects

Overall, CBZ is generally well tolerated. Short-term treatment adverse effects that have been reported include sedation, ataxia, dizziness, nausea, vomiting, constipation and diarrhea. Long-term treatment with CBZ may modify plasma lipids, change the concentration of sex hormones, produce hyponatremia, increase appetite and cause weight-gain, reduce the number of white blood cells and induce several allergic reactions.[157]

PK drug interactions involve both CBZ metabolism and CBZ binding to serum proteins. CBZ not only induces the capacity of CYP3A4 to enhance its own metabolism, but increases this enzyme system's capacity to metabolism endogenous molecules and numerous drugs that are biotransformed through this mechanism.[156] Depending on the concomitant medication, CBZ plasma concentration will be increased or decreased. Further details of the known interaction are available elsewhere.[2,144]

Ethosuximide

Ethosuximide is a succinimide anticonvulsant that is used mainly for absence seizures particularly when more effective agents fail or are not tolerated. Other seizure types that may be favorably influenced by ethosuximide include atypical absence and myoclonic seizures.[2]

Mechanism of Action

The mechanism of action of ethosuximide has not been identified. However, the hypothesis is that ethosuximide blocks thalamic ’T’ type calcium channels, an effect possessed by drugs that are effective against absence seizures.[156] Ethosuximide either reduces the number or the conductance of these channels.[2] Other studies in thalamocortical neurons of rats have demonstrated an inhibitory effect of ethosuximide on non-inactivating sodium currents and on calcium-dependent potassium channels, but not on “T” calcium channels.[2]

Adverse Effects

Ethosuximide is relatively safe and effective. Skin rashes and allergic and hypersensitive reactions are not common.[156] Gastrointestinal side effects are the most common adverse reaction. They include mostly abdominal discomfort, vomiting, diarrhea, and hiccups. Overdose may cause sedation and altered behavior. Little information is available regarding the drug’s teratogenicity. Ethosuximide exhibits relatively few drug-drug interactions but enzyme-inducing drugs can increase the clearance of ethosuximide or valproate can inhibit the metabolism.[2]

Felbamate

After being approved felbamate was found to cause severe bone marrow and liver toxicity. Given this toxicity, the main indication for felbamate currently is used as a third or fourth line therapy in children with Lennox-Gastaut syndrome and similar forms of epilepsy who failed to respond to other AEDs.[2,158]

Mechanism of Action

Felbamate has been proposed to have a unique dual mechanism of action as a positive modulator of $GABA_A$ receptors and as a blocker of NMDA receptors, particularly isoforms containing the NR2B subunit.[159] Felbamate has been shown to inhibit the NMDA-induced intracellular calcium currents.[2] Although there is some evidence that felbamate affects the GABA receptor there are contradictory studies on the lack of effect on ligand binding to the $GABA_A$ receptor and potentiation of GABA responses at high felbamate concentrations.[2]

Adverse Effects

The most common side effects in children are anorexia, somnolence, insomnia, vomiting, and nausea.[158] However, during the first year following approval it was revealed that felbamate was associated with a relatively high incidence of life-threatening side effects. These included aplastic anemia and hepatotoxicity.[2,4] Aplastic anemia was not reported in any child younger than the age of 13 years.[2]

Gabapentin

Gabapentin (GBP) has approval for adjunctive therapy of partial seizures in children 3 years of age or older.[2,144]

Mechanism of Action

GBP was designed as a GABA analog, and some studies have suggested that it modulates the action of the GABA synthetic enzyme, glutamic acid decarboxylase (GAD) and the glutamate synthesizing enzyme, branched-chain amino acid transaminase, resulting in increased GABA synthesis.[159] GBP increases non-synaptic GABA responses from neuronal tissues in vitro and increases GABA levels in brain.[159] While GBP was not found to have significant effect on any calcium channel current subtype (T, N, or L), studies have demonstrated that the drug may act by binding to the $alpha_2$delta1 subunit of the calcium channel presumably inhibiting excessive neurotransmitter release by interfering with calcium channel functional expression or trafficking.[2,159]

Adverse Effects

Adverse effects with GBP are uncommon in children but typical side effects are somnolence, dizziness, ataxia, fatigue, nystagmus, headache, tremor, diplopia, and nausea and vomiting.[2]

Lamotrigine

Lamotrigine (LTG) was approved in 1998 as adjunctive therapy in the treatment of partial seizures and patients with LGS $\geq$ 2 years of age.[2,158] It has since been approved for monotherapy adults (aged 16 years and older) with partial-onset seizures who are receiving treatment with carbamazepine, phenytoin, phenobarbital, primidone, or valproate as the single antiepileptic drug (AED) and bipolar disorder.

Mechanism of Action

LTG appears to have a broad-spectrum of action; however, the identified mechanisms do not fully explain its clinical effectiveness. Studies have demonstrated the LTG blocks sodium channels, leading to stabilization of neuronal membranes.[2] It has also been suggested that LTG reduces the effects of glutamate on the rat's spinal cord and reduced GABA receptor-mediated neurotransmission in rat amygdala, suggesting that a GABAergic mechanism may also be involved.[2,160]

Adverse Effects

The toxicity profile of LTG includes common adverse events seen with other AEDs, including dizziness, diplopia, headache, ataxia, blurred vision, nausea, somnolence, and vomiting.[2] The FDA carries a black-box warning for severe skin rashes, and in clinical trials, serious rashes resembling Stevens-Johnson syndrome occurred in about 1 in 300 adults and 1 in 100 children.[158]

Levetiracetam

Levetiracetam (LEV) is an anticonvulsant medication available since 2000 and is indications include monotherapy and as add-on therapy in partial-onset seizures as well as in patients with idiopathic generalized epilepsy for myoclonic and generalized tonic-clonic seizures in adults and children $\geq$ 4 years of age.[161] It is widely used in patients younger than 4 years as monotherapy and in other epilepsy types.[2,144] It has been shown to have good efficacy and an excellent safety profile in adults and older children.

Mechanism of Action

The exact mechanisms of action of LEV are not completely understood. It is thought the drug binds with high affinity to the synaptic vesicle protein SV2A, a protein thought to be involved in the regulation of synaptic vesicle fusion, exocytosis, and neurotransmitter release.[161] LEV alters epileptiform burst firing but not normal neuronal excitability.[161]

Adverse Effects

LEV is a popular choice for pediatric epilepsy given that it has no known interaction with other AEDs and is well tolerated. The most common adverse events reported were headache, infection, anorexia, and somnolence. However, occurrence of emergent behavioral side effects has been described. Specifically, aggression, emotional liability, oppositional behavior, and psychosis has been described in some children.[2]

Phenobarbital

Phenobarbital (PB) is a barbiturate and one of the oldest anticonvulsants and sedatives, have been first synthesized in 1902.[162] It has been useful in the management of severe and persistent seizures that do not response to more routine anticonvulsant therapy.[2,144] It is the first-line treatment for neonatal seizures.[16] Neonatologists use this drug because it is a well-known anticonvulsant available as injectable formulation and has been standard of care for decades.

Mechanism of Action

Barbiturates enhance GABA-mediated inhibitory synaptic transmission on $GABA_A$ receptors.[163] Current views suggest the phenobarbital modulates the postsynaptic effect of certain neurotransmitters. The modulation is thought to affect both the inhibitory substance GABA and excitatory amino acids such as glutamate.[2]

Adverse Effects

The most frequently encountered adverse effects are sedation, disturbance of mood and behavior (excitement, irritability, aggression, or confusion) and induction of hepatic metabolism.[2,144] Changes in metabolism can have an effect on the disposition of a wide variety of other drugs. In addition to frequent adverse effects, phenobarbital can cause serious adverse effects and interact with other medications administered in the neonatal intensive care unit (NICU).[164] It has been shown to induce apoptosis and cellular reorganization in neonate animals at concentrations obtained with clinical use in babies.[165,166] It is also suggested that phenobarbital can inhibit cell proliferation by over 60% in animal models.[166] Exacerbation of seizures may occur with discontinuation of phenobarbital maintenance therapy.

Phenytoin

Phenytoin (PHT) is also an older anticonvulsant with FDA approval in 1939. It has been shown to be effective for status epilepticus, partial seizures, tonic seizures, partial seizures secondarily generalized, and generalized tonic-clonic seizures.[2,144] PHT is

widely used to treat neonatal seizures.[16] It was shown to provide effective treatment for epilepsy without causing sedation, a major advantage over phenobarbital.[156]

Mechanism of Action

PHT has been suggested to act by reducing electrical conductance among brain cells by stabilizing the inactive state of voltage-gated sodium channels and delaying its rate of recovery from inactivation.[156,163] This will block frequency, use, and voltage-dependent neuronal sodium channels, thus limiting the repetitive firing of action potentials.

Adverse Effects

PHT can affect the central nervous system and can include nystagmus, incoordination, and ataxia. Somnolence and mood changes, nausea, extrapyramidal abnormalities, cerebello-vestibular toxicity, and mental changes have been reported.[156] Similar to phenobarbital, phenytoin can induce apoptosis, inhibit cell proliferation, and cellular reorganization in neonate animals.[165,166] Rarely, skin rashes, hypersensitivity, lymphoma, and hepatotoxicity have been reported.[156]

Valproate

Valproic acid (VPA) was approved in 1978 for monotherapy and adjunctive therapy of complex partial seizures and simple and complex absence seizures. It rapidly became the drug of choice because it was the first drug with a broad spectrum of activity against different seizure types.[156] It is indicated for monotherapy and adjunctive therapy in the treatment of patients with complex partial seizures and absence seizures. VPA has found

some effectiveness in the treatment of partial seizures, LGS, infantile spasms, neonatal seizures, and febrile seizures.[2,144]

Mechanism of Action

The precise mechanism of action for antiepileptic effect is unknown. VPA increases the brain GABAergic, inhibitory activity.[2,156] In addition, VPA reduces cortical excitability by reducing sustained repetitive high-frequency firing by blocking voltage-sensitive sodium channels or by activating calcium-dependent potassium conductance.[2,156]

Adverse Effects

Gastrointestinal effects, hair loss, and weight gain have been reported, but much more important is the finding of teratogenicity associated with valproate which is dose-dependent and may be associated with severe birth defects, such as spina bifida, cardiac abnormalities, skeletal, and renal malformations, as well as a decline in cognition, IQ, language, and possible autism.[156] Severe hepatotoxicity has also been reported and children younger than 2 years old are at a higher risk. In addition, there is an increased risk of liver failure and death from liver failure in patients who have a genetic liver problem caused by mitochondrial disorder.

Marijuana

The use of medical marijuana for childhood epilepsies has gained popularity in the recent years. There are three compounds that have been isolated and identified as the most

potent: D9 -tetrahydrocannabinol (THC), cannabidiol (CBD) and cannibinol. CBD is the non-psychotropic compound which has shown promise as an anticonvulsant.[167]

Only two small (N=20) double-blinded, placebo-controlled studies of cannabinoids in epilepsy have been published.[167] In both studies, daily oral CBD was used as an adjunct to antiepileptic drugs. One study demonstrated a beneficial effect in seven out of eight patients, while the other showed no improvement in 12 patients.[167] A Cochrane database review concluded that no reliable conclusions could be drawn regarding the efficacy of cannabinoids as a treatment for epilepsy.[168]

Mechanism of Action

CBD interacts with many non-endocannabinoid signaling pathways. It is a blocker of the equilibrative nucleoside transporter (ENT), the orphan G-protein-coupled receptor GPR55, and the transient receptor potential of melastatin type 8 channel.[169] CBD also enhances the activity of the 5-HT receptor, the alpha3 and alpha1 glycine receptors, the transient receptor potential of ankyrin type 1 channel, and has a bidirectional effect on intracellular calcium.[169] Its precise anticonvulsant mechanism is unknown.

Adverse Effects

Multiple small studies of CBD safety in humans in both placebo-controlled and open trials have demonstrated that is well tolerated across a wide dosage range. No significant central nervous system side effects were seen in acute and chronic administration.[169]

These studies were completed in adults and the toxicity in children is not well understood.

2.3 OTHER THERAPIES

2.3.1 The Ketogenic Diet

The ketogenic diet (KD) is a high-fat, adequate-protein, low-carbohydrate regimen that is used primarily to treat refractory epilepsy in children. The primary indications for KD is first-line therapy for the treatment of seizures in association with glucose transporter protein deficiency and pyruvate dehydrogenase deficiency.[2] KD may be an alternative treatment, usually after the failure of valproate, for generalized epilepsies, particularly those with myoclonic seizures, including Dravet syndrome and Doose syndrome.[2,170]

Mechanism of Action

The production of ketone bodies from the oxidation of fat is the primary source of metabolic energy, which appears to be involved in the control of seizures.[171] A recent review discussed in detail the neurochemical mechanisms of the KD that have been studied, although, the mechanism on anticonvulsant effect is unclear. Ketone bodies derive from the metabolism of nonesterified fatty acids. They are released from adipose tissue in response to a decrease in blood glucose, such as that which occurs during fasting.[171]

Similar mechanisms are involved in the KD, during which long-chain fatty acids are metabolized in the liver and converted into ketone bodies. These fatty acids are oxidized

in the mitochondria, producing high levels of acetyl-CoA, which cannot be oxidized in the Krebs cycle. The excess acetyl-CoA is converted to acetoacetate and subsequently to acetone and β-hydroxybutyrate.[171] The ketone bodies cross the blood-brain barrier and are transported by monocarboxylic acid transporters to the brain interstitial space, the glia and the neurons. In these tissues, the ketone bodies act as substrates in the Krebs cycle and respiratory chain, contributing to brain energy metabolism.[171]

The implication of these mechanisms is that glycolysis and glucose flux are reduced, tricarboxylic acid cycle is increased, and cerebral energy reserves are increased.[2] These biochemical changes appear to favor cerebral energetics and lead to increased resistance to seizures and a possible favorable cognitive effect.[2]

Adverse Effects

The diet can cause nausea and vomiting, fatigue, loss of appetite, and hypoglycemia.[2,172] Severe adverse effects are pancreatitis, death, and coma. Some long-term effects that have been reported of the diet include kidney stones, skeletal fractures, and slow growth. (grosebeck, 2006, developmental medicine and child neurology).

Modified Atkins Diet for Seizures

In addition to KD, a modified Atkins diet can be used for intractable epilepsy. It is similar to the KD in there is a higher proportion of fat and a lower proportion of carbohydrates compared with that in regular diets. The use of the Atkins diet has not

been well studied. A study evaluated the use of this diet in 20 patients and it was shown to be effective and well tolerated.[2]

2.3.2 Surgery

Surgical treatment is used for children with medically resistant seizures.[2] This treatment involves removal of an area of the brain with an aim of alleviating seizures. This can be applied to various brain structures or to cranial nerves. Extensive evaluation needs to be determined whether seizure onset can be localized and to establish intractability. Surgery does not need to be considered a last resort as the rate of cognitive and behavioral comorbidity associated with early onset of epilepsy is a high and early cessation of seizures likely leads to improved neurobehavioral outcomes. In a recent Monte Carlo simulation using parameter from the literature epilepsy surgery yielded a higher life expectancy than continued medical treatment for surgically eligible children.[173]

Surgical resection is offered to suitable candidates with drug-resistant focal epilepsy, about half will have long-term postoperative freedom from seizures.[174] The goal for respective epilepsy surgery is the identification and accurate localization of the epileptogenic zone. The history of disabling medically refractory focal seizures needs to be clearly established, which requires at least a good description of seizure semiology, repeated interictal EEG data, and an optimal MRI scan before consideration for surgical treatment.[4] MRI is the choice for evaluating pediatric epilepsy surgery candidates but functional imaging, PET, MRS, single-photon emission computerized tomography, and fMRI each have their place in identifying dysfunctional cortical regions. An additional

aspect of presurgical evaluation is extensive EEG recordings and neurobehavioral assessments.

Epilepsy surgery has an important role in the treatment of pharmacoresistant epilepsy. It should be considered on a case by case basis and after careful evaluation.

2.3.3 Therapeutic Hypothermia

Therapeutic hypothermia was first used in the 1950s to prevent ischemic brain injury during cardiac surgery and its use has increased to include various neurological conditions, including traumatic brain injury, stroke, and anoxic brain injury resulting from cardiac arrest.[175,176] It is a common treatment for neonatal seizures. Therapeutic hypothermia is thought to be neuroprotective and reduce brain injury. It reduces cerebral metabolic rate and oxygen demand thereby decreasing cerebral edema and an intracranial pressure which helps preserve integrity of blood brain barrier by stabilizing lipoprotein membranes.[177]

The discussion of therapeutic hypothermia is relevant to this thesis as it is a common treatment for neonatal seizures. If a DBS assay is used for TDM or defining PK parameters in neonates, it will be useful to know how therapeutic hypothermia will affect physiology and pharmacokinetic parameters.

Definition and Methods of Hypothermia

Body temperature is usually maintained near a constant level of around 37°C. Hypothermia is when the core body temperature drops below the required temperature for normal metabolism and body functions which is defined as 35°C. Therapeutic hypothermia is a medical treatment that lowers a patient's body temperature in order to help reduce the risk of ischemic injury to tissue following a period of insufficient blood flow. Therapeutic hypothermia has been defined into three categories, depending on depth of cooling: mild, moderate, and deep (Table 11).[178]

Table 11: Categories of Therapeutic Hypothermia

Category	Core Temperature (°C)	Group
Mild	32-35	Adults, children, and neonates
Moderate	28-32	Adults, children, and neonates
Deep	<28	Neonates

The medical methods by which hypothermia is induced falls into two categories: invasive and non-invasive. Invasive methods could include cold IV infusion or cooling catheters while non-invasive would be cooling blankets or fans. Cooling catheters are inserted into the femoral vein. Cooled saline solution is then circulated through the body and cools the patient's whole body by lowering the temperature of the patient's blood. Catheters reduce body temperature at rates ranging from 1.5°C - 4°C per hour. Catheter-based temperature management has been shown to provide faster, more precise and more efficient cooling compared to all external methods, especially conventional.[179,180] Adverse events associated with cooling catheters include bleeding, infection, vascular puncture, and deep vein thrombosis.[181]

Cooling blankets or fans are an inexpensive and easy method for cooling patients. Cooling is slower than invasive methods at a range of 0.5°C - 1°C per hour. For cooling blankets, cold water is circulated through a blanket wrapped around the torso or leg. Cooling blankets lower a patient's temperature exclusively by cooling a patient's skin and accordingly require no clinician performed invasive procedures. Water blankets could overshoot the desired temperature, have slower induction time, increased compensatory response, and decreased patient access. There are also a number of non-invasive head cooling caps designed to target cooling at the brain. These caps are filled with a coolant agent such as ice or gel.

Animal and Human Studies

In addition to physiological changes, new evidence from animal studies suggests hypothermia may be effective in various clinical situations. Experiments have been conducted using different species, models, degrees and duration of cooling. Post-hypoxic hypothermia has been shown to be neuroprotective in the neonatal rat, newborn pig, and near term fetal sheep.[182-184] Based on these studies, cooling should last for 72 hours and cooling should start with a maximum delay of 5.5 h after birth.[185,186] These observations have been confirmed by clinical studies.

In two multi-center, randomized, controlled trials of hypothermia treatment increased the odds for survival and better outcomes in neonates treated with hypothermia (33°C).[187,188]

Whole-body hypothermia reduced the risk of death or disability in infants with moderate or severe HIE.[189] Varies clinical trials have used different methods of cooling (cooling fan, head cooling, whole-body cooling) and shown that each is effective in reaching the target rectal temperatures.

Physiologic Changes

Cardiovascular

During hypothermia, there is a redistribution of blood away from the gastrointestinal tract, kidney, and liver towards coronary and cerebral circulation. Blood flow to the organs reduces with progressive hypothermia. The heart, liver and brain maintain a higher level of flow in comparison with the kidneys to 27°C. However, at 25° to 20°C renal and myocardial blood flows are sustained at 20% to 25% of normothermic levels whereas cerebral and hepatic blood flow show greater decreases.[190,191]

In neonates undergoing therapeutic hypothermia, heart rate drops by 14 to 45 beats per minute and returns to normal after rewarming.[189,192-194] Cardiac output was also reduced by 33% and stroke volume by 23% in neonates undergoing moderate hypothermia.[195] For moderate hypothermia, there was no change in ECG, blood viscosity, or fibrillations when cooled to 33°C.

Neurological

Studies have suggested that cerebral blood flow is decreased in hypothermia. Specifically, cerebral blood flow decreased 5% for every 1°C in canines.[196] In adult

animals and humans, EEG parameters of amplitude and frequency decrease at <32°C. However, one study evaluated whether core body temperature affected quantified EEG parameters during the rewarming phase of hypothermia treatment in neonates with HIE. They found that core body temperature had no significant effects on the EEG parameters.[197] There is no evidence to suggest a difference in seizure incidence during hypothermia in either adults or neonates.

Hematologic

An association between abnormal bleeding and hypothermia is well documented in the literature.[198] The coagulation cascade consists of a series of enzymatic reactions. A research group demonstrated an increase in the activated partial thromboplastin time from a mean $\pm$ SD of 36 $\pm$ 0.7 seconds at 37°C to 46.1 $\pm$ 1.1 seconds at 31°C in samples from 10 volunteers.[199] Hypothermia has been shown to inhibit platelet function. An in vivo study found that thromboxane-A_2 and thrombin-induced platelet aggregation were inhibited during hypothermia in healthy volunteers.[200] Platelet dysfunction was reversed with rewarming. Decreased platelet count was observed in 10 healthy volunteers during hypothermia.[199] Mild thrombocytopenia was present in neonates at temperatures below 35°C.[201] In both adults and neonates these studies did not find an increase in bleeding time or hemorrhage.

Endocrine and Metabolic

As with a decreased blood flow, basal and cerebral metabolic rates are decreased during hypothermia. In one study examining newborn swine, there was a 5-7% decrease for

every 1°C drop in body temperature.[202] Decreased CO_2 and O_2 production have been observed during cooling. There is also an increase in lipolysis.[203] Hypothermia can cause hyperglycemia, which occurs rapidly with cooling, and it is associated with decreases in both pancreatic release of insulin and insulin sensitivity.[198] Cooling results in increased gluconeogenesis and glycogenolysis, which may be due to a rise in circulating catecholamines and cortisol.[204] Hyperglycemia has been associated with worse neurological outcomes.[205] Frequent monitoring of glucose, lactate, and oxygen levels are particularly important during rewarming.

Respiratory

Blood gas values are affected by hypothermia because of increased solubility of gases at lower temperatures and reduced metabolic rate.[192] The blood pH increases by 0.016 points with every 1°C drop in core temperature while the CO_2 partial pressure decreases.[192,206] The oxyhemoglobin dissociation curve is shifted to the left at lower temperatures, resulting in a higher affinity of hemoglobin for oxygen. The resulting decrease in oxygen release to tissues is counterbalanced by decreased tissue oxygen demand at lower temperatures.[207] Pulmonary vascular resistance may increase at lower temperatures which are most likely due to vasoconstriction. As with other body functions, the respiratory rate is decreased at lower temperatures.

Renal Function and Electrolyte Levels

Although animal models and studies in adults report alterations in renal perfusion and renal function during hypothermia, randomized clinical trials of hypothermia in neonates

with birth asphyxia have not found significant differences in urine output or creatinine.[192,201] It is known that potassium moves into cells as temperature drops. Patients may experience mild hypokalemia. Adults studies have suggested that there is increased electrolyte loss and hypomagnesaemia, hypocalcaemia, and hypophosphatemia can occur.[208] No significant electrolyte disturbance have been reported in randomized clinical trials of neonates.[201]

Musculoskeletal

The body's response to hypothermia will involve various mechanisms to generate heat. Changes in the musculoskeletal system start at just a 1°C drop in core temperature.[192] Shivering, the major musculoskeletal change occurs between core temperatures of 34 – 36 °C and diminishes with core temperatures below 34°C.[198] It is suggested that shivering occurs when the preoptic region of the hypothalamus is either cooled or reset to a higher baseline temperature.[209] This activation results in an integrated series of responses that activates the α motor neurons in an involuntary, oscillatory muscular activity that augments metabolic heat production, resulting in increased body temperature.[198]

Shivering has also been shown to increase the levels of norepinephrine and cortisol and increase the basal metabolic rate.[192] This results in increases in oxygen consumption. These adverse effects cause stress on the body and can take away from the beneficial aspects of hypothermia. Thus, administration of sedatives and analgesics is recommended to be given to patients undergoing therapeutic hypothermia.

Table 12: Physiologic Effects of Mild to Deep Hypothermia in Neonates

Physiological	Effect
Cardiovascular	Decreased heart rate Decreased cardiac output Vasoconstriction Increased blood pressure
Endocrine	Decreased insulin release and sensitivity Increased gluconeogenesis and glycogenolysis Increase in catechol and cortisol
Hematologic	Mild thrombocytopenia
Metabolic	Decreased basal and cerebral metabolic rate Decreased CO_2 and O_2 production Increased lipolysis
Neurologic	Decreased cerebral blood flow
Renal	No change in urine output No change in electrolyte alterations
Respiratory	Increase blood pH Decreased respiratory rate Increase in pulmonary vascular resistance

Pharmacologic Changes

Pharmacokinetics

Considerations for hypothermia therapy in neonates have not been well studied. Few studies have examined the changes in drug disposition. Table 13 summarizes pharmacologic changes during hypothermia. Many PK parameters can be affected by hypothermia including absorption, distribution, elimination, and metabolism.

Table 13: Pharmacologic Effects of Mild to Deep Hypothermia

Pharmacological	Effect
Absorption	Decreased gastrointestinal motility May cause prolonged time to reach maximum concentration
Distribution	Increased or decreased volume of distribution; affected by physiological factors Changes in protein binding capacity, lipid solubility, and tissue binding capacity
Metabolism	Decreased bile flow Decreased activity of active hepatobiliary transport Reduced enzyme metabolic activity Changes in binding pocket conformation Decreased rate of redox reactions Change in affinity of oxygen for ferric iron Changes in nicotinamide adenine dinucleotide phosphate P450 reductase activity Location of metabolic process Membrane fluidity
Elimination	Decreased renal perfusion Changes in tubular secretion and reabsorption unknown
Pharmacodynamics	Enzymatic inactivation or reduction Drug-target affinity Onset and recovery time of the effect

Absorption

Changes in absorption may cause prolonged time to reach maximum concentrations and decreased gastrointestinal motility. These changes will be affected by physiological factors such as gastric and duodenal pH, acid dissociation constant (pK_a), and lipid solubility. If the therapy is given orally it may take longer to be effective. The extent of absorption has not been studied.

Distribution

Distribution can be decreased or increased depending on the physiochemical properties of the drug. It will be affected by physiological factors such as blood perfusion and pH. Changes in these factors can change the protein binding capacity, lipid solubility and tissue binding capacity. During hypothermia there is a redistribution of blood away from the GI tract, kidney, and liver towards coronary and cerebral circulations. This could lead to more drugs crossing the blood brain barrier causing an enhanced cerebral effect. Drugs administered before, may be extensively distributed and remain stored in the tissues during hypothermia. During rewarming, drugs may redistribute from tissues, leading to a rise in plasma levels that may have a clinical effect. A study examined the effect of hypothermia on gentamicin in piglets. The serum concentration of gentamicin was consistently higher during hypothermia than during normothermia. Elimination half-life was longer, volume of central compartment, volume of distribution, and total body clearance were significantly reduced during hypothermia.[210]

Metabolism

Bile flow and activity of active hepatobiliary transport decrease during hypothermia. Studies have examined hepatocyte enzymatic function and found reduced enzyme metabolic activity, changes in binding pocket conformation with reduced substrate affinity, decreased rate of redox reactions, changes in affinity of oxygen for ferric iron, location of metabolic process, and membrane fluidity.[211] Studies in piglets and neonates

have shown increased plasma concentrations of fentanyl and morphine suggesting decreased CYP3A and UGT activity.[212,213]

Elimination

During hypothermia, renal elimination appears to decrease. Nishida et al investigated phenolsulfonphtalein (PSP) disposition in male Wistar rats during hypothermia. PSP, a hydrophilic dye used as a renal function test compound in humans, and is excreted into the bile and urine as a free form or conjugative metabolite in rats.[214] The plasma disappearance of free PSP after IV administration was delayed in hypothermic rats. Total clearance decreased to about 60% and 40% that of normothermic rats.[214] The decrease in clearance was suggested to be due to decreased cardiac output.

Pharmacodynamics

Pharmacodynamic changes can also occur during hypothermia. Enzymatic inactivation such as changes in EC_{50}, drug-target affinity, and onset and recovery time of the effect could be affected. One report described a 120% increase in the duration of action of vecuronium in hypothermic subjects compared to normothermic subjects.[215]

2.4 SUMMARY AND RATIONALE FOR MY PROJECTS

Topiramate has the potential to be an effective therapy for neonatal seizures and Dravet's syndrome. As has been discussed in this chapter, a limiting factor in the further development of TPM for these conditions is obtaining the necessary PK and pharmacodynamic information to design adequately controlled trials. One major hurdle

in addressing this problem is the severe restriction on blood sampling in critically ill children, in whom blood is collected for a multitude of lab tests. This circumstance reduces the blood volume available for pharmacology studies. Consequently, the necessary research on PK and dose finding is often limited.

One solution to this problem is the use of whole blood or DBS to measure drug concentrations in replace of plasma. This approach reduces the volume of blood needed for a drug assay by greater than 50%. Further complicating the use of whole blood for TPM measurements is the fact that the drug exhibits saturable binding to carbonic anhydrases in RBCs. As a result, there is a non-linear relationship between whole blood and plasma, which complicates interpretation and comparison of TPM concentrations between matrices.[93] If TPM is measured in whole blood without correction for non-linear binding this would result in inaccurate PK estimates. For example, Filippi et al used DBS to measure TPM concentrations in neonates.[109] Blood values were corrected by the hematocrit to obtain plasma values using an equation that assumed TPM is isolated in the plasma. However, this equation doesn't take into account the binding of TPM to carbonic anhydrase or TPM present in the RBCs. This equation would result in concentrations higher than what would be seen if correcting for TPM binding to carbonic anhydrases. It is important to establish the relationship between whole blood and plasma concentrations due to the binding. With an improved assay we could quickly measure TPM concentrations in children while limiting the volume of blood that is collected. Thus, my thesis project characterized the relationship between whole blood or DBS and plasma to enable use of a DBS assay and method to account for the nonlinear binding to

provide a more accurate comparison of topiramate concentrations in whole blood to plasma concentration, which is the matrix typically measured in the clinic. This will be important for clinical use and characterization of TPM's disposition. If TPM is measured in whole blood or DBS without correction for the non-linear binding, you will not be able to relate that concentration to a plasma concentration as it is not a one to one relationship.

From September 2010 to May 2015, I conceptualized, managed, and coordinated the characterization of the relationship between whole blood and plasma TPM concentrations. There are 3 components to my project with an overall objective of characterizing the relationship between plasma and whole blood topiramate concentrations for use in a clinical setting.

2.4.1 Hypothesis and Specific Aims

The overall hypothesis for this project is that a physiological-based equation will accurately estimate plasma concentrations based on a whole blood or DBS concentration. In order to characterize the processes necessary to determine an appropriate equation that can capture the relationship DBS, whole blood, and plasma assays were developed. To further examine the relationship between whole blood and plasma concentrations three studies were completed in dogs, adults, and pediatrics.

Aim #1: Develop assays to measure TPM concentrations in DBS, whole blood, and plasma in a small volume of sample to accommodate the lower number of blood samples available in pediatric patients and animals.

Aim #2: Characterize the relationship between whole blood and plasma TPM concentrations and compare the PK of IV and oral TPM in plasma and whole blood in dogs with naturally-occurring epilepsy.

Aim #3: Characterize the relationship between whole blood and plasma TPM concentrations in adults and children.

2.4.2 My Role in Each Project

Development of Bioanalytical Methods for Measurement of Topiramate Concentrations

- Developed and validated an assay for measurement of TPM in whole blood, plasma, and DBS according to the recommended guidelines provided by the US Food and Drug Administration.[216]

Whole Blood TPM Pharmacokinetics for Intravenous and Oral Dosing in Dogs with Naturally-Occurring Epilepsy

- Performed LC-MS assay of topiramate in whole blood
- Performed PK analysis of dog whole blood concentration-time data
- Wrote final study report and manuscripts

Comparison of Whole Blood and Plasma Assay Methods for Measurement of Topiramate Concentration in Adults

- Designed the study and wrote the protocol

- Prepared applications and obtained IRB approval
- Served as principal investigator and coordinator (recruited, screened, enrolled, and consented subjects)
- Performed LC-MS assay of topiramate in whole blood, plasma, and DBS
- Wrote final study report and manuscripts

Comparison of Topiramate Concentrations in Whole Blood and Plasma in Children

- Co-principal investigator and coordinator
- Designed the study and wrote the protocol
- Prepared applications and obtained IRB approval
- Coordinated meetings and presented study to investigators at Gillette Children's Specialty Healthcare, MINCEP, and Minnesota Epilepsy Group
- Recruited subjects from Minnesota Epilepsy Group Clinic, MINCEP, and Gillette Children's Specialty Healthcare
- Wrote final study report and manuscripts

CHAPTER 3: DEVELOPMENT OF BIOANALYTICAL METHODS FOR MEASUREMENT OF TOPIRAMATE CONCENTRATIONS

3.1 INTRODUCTION

AEDs are the mainstay for the control of seizures in the management of epilepsy.[217] The goal of AED therapy is long-term seizure control with minimal or no side effects. However, fluctuations in AED concentrations may increase the risk of breakthrough seizures at lower plasma concentrations and adverse events may occur at peak plasma concentrations.[218,219] In practice it is the patient who is treated and not the concentration. The dose of a drug is adjusted, using an individual's drug concentration as a guide, to optimize a drug's efficacy, avoid, minimize or identify toxicity, and detect or confirm poor compliance (Table 14).[220]

It is therefore important to appreciate that therapeutic drug monitoring (TDM) begins before a drug concentration is measured and that the measurement itself is only part of the overall process of planning, monitoring, and optimally adjusting the dosage regiment.[220] TDM can guide dosage adjustments and is especially useful for children due to the greater PK variability in this population compared to adults.[218] TDM is also important in assessing compliance versus drug adherence with the prescribed regimen.[218,221,222] Side effects in particular can lead to treatment non-adherence,[223,224] a significant problem in patients with epilepsy with a prevalence as high as 40%.[225] Periods of poor adherence to AED therapy can result in undesirable clinical consequences, such as increased seizure occurrence and higher incidences of emergency department visits, hospital admissions, motor vehicle injuries, fractures, and death, when compared with periods of compliant dosing.[225-227]

There are two types of concentration ranges that can be defined for a compound. First, the reference range can be defined as a range of a drug concentration, which specifies a lower limit below which a therapeutic response is unlikely to occur and an upper limit where toxicity is more likely to occur. Second, the suggested therapeutic drug concentration range refers to a range of concentrations where a majority of the population is thought to experience efficacy with a low number of adverse events. A reference range for TPM has been established (5-20 mcg/ml).[218] In addition, one study has examined the therapeutic concentration response in sixty-five patients with refractory focal epilepsy.[219] They found optimal treatment response higher than 2 mcg/ml but no further increase in efficacy occurred at concentrations above 10.5 mcg/ml.[219]

Table 14: General indications for therapeutic drug monitoring[220]

#	Indication
1.	After initiation of treatment (to provide a baseline steady-state concentrations)
2.	After change in drug dosage, in particular when non-linear kinetics apply (to confirm new drug concentration)
3.	At therapeutic failure (to confirm or exclude a pharmacokinetic explanation for uncontrolled seizures or adverse events)
4.	To establish an individual therapeutic concentration range (when a person has attained the desired clinical outcome), which can be used subsequently to assess potential causes of change in response
5.	To identify or control for drug-drug interaction
6.	After a change in drug formulation (including generic substitution)
7.	To guide dosage adjustment in patients where potentially important pharmacokinetic changes are anticipated consequent to physiological or pathological changes (e.g. pregnancy, hepatic disease, renal disease, and gastrointestinal conditions)
8.	When poor drug adherence is suspected
9.	Suspected toxicity
10.	To guide dose adjustment for AEDs with dose-dependent pharmacokinetics
11.	An emergency situation (suspected overdose, status epilepticus)

3.2 BIOLOGICAL FLUIDS FOR DRUG MEASUREMENT

AED concentrations are commonly measured in plasma or serum. Concentrations are usually identical in these matrices, and most published data relate to concentrations in these fluids.[228] There are a number of different matrices that can be used to measure drug levels including serum, plasma, saliva, whole blood, tears, urine, and cerebrospinal fluid.

DBS is a technology that uses whole blood and has been used to determine drug concentration for preclinical and clinical studies. It has been determined to be feasible for a wide range of compounds. DBS technology has a number of advantages over conventional sample collection. These include practical, clinical, and cost considerations. Use of DBS technology allows for less invasive sampling (finger or heel prick rather than a venipuncture) and as much as a 90% reduction in blood volume. Use of DBS can simplify sample collection and make shipping of samples easier.

A general guideline for blood collection in children used by many Institutional Review Boards (IRB) is a maximum of 3-5% of blood volume over a one to three month period for research purposes.[229] This restriction leaves a limited amount of blood volume for PK studies. A whole blood or DBS assay would be desired for drug monitoring in children and infants as the volume that can be collected is often limited.

It is practical to measure drug concentrations in whole blood or DBS, but drug concentrations may not be identical in RBCs and plasma as the composition and complexity of the different matrices is significantly different from each other.[228,230] The

nature and chemical properties of the drug can determine the matrix for measurement as some drugs may bind to different constituents in the blood. Understanding the interactions between analyte and matrix aids in the decision of a suitable sample preparation technique.[230] When working with whole blood or DBS samples, the distribution of the compound between RBCs and plasma, the enzymatic stability of the analyte in blood, and protein and RBC binding should be considered.[230]

Analysis of whole blood data may be misleading if the concentration of drug differs between plasma and whole blood as a consequence of differential binding to a specific component in the blood. In addition, the blood to plasma ratio determines the concentration of the drug in whole blood compared to plasma and provides an indication of drug binding to erythrocytes. Knowledge of the RBC partitioning of topiramate into RBCs enables a rational choice of appropriate biological fluid and physiologically meaningful referencing of PK parameters of drugs to concentrations in whole blood, plasma, or DBS.

3.3 SIGNIFICANCE AND AIMS OF CHAPTER

The use of whole blood or plasma does not result in comparable TPM comcentrations. TPM exhibits saturable binding to carbonic anhydrase in RBCs and the use of DBS sample collection to measure this compound.[93] TPM concentrations are expected to be higher in whole blood concentrations versus plasma concentrations, particularly at TPM concentrations near the lower limit of its reference range (5-20 mg/L).[218] Thus, TDM of TPM using whole blood samples may mislead the clinician, who is accustomed to

receiving TPM concentrations measured in plasma samples. The differences in whole blood concentrations may result in different PK parameter estimates.

3.3.1 Aim

The primary objective of this chapter is to develop an assay for measurement of TPM concentrations in whole blood and DBS.

The specific aim of this project is to:

Aim #1: Development of a sensitive method for measuring TPM concentrations in whole blood following DBS sample collection.

3.4 TOPIRAMATE ASSAYS

Several methods have been described to measure TPM in plasma or serum.[231-235] Gas chromatography tandem MS was used in the early development of TPM.[236,237] Fluorescence polarization immunoassay (FPIA) is another commonly used method.[238] FPIA is reportedly highly accurate and precise, but may be prone to interference from metabolites.[238] LC-MS is a highly sensitive, accurate and precise method of quantitating drugs and metabolites in biological fluids.

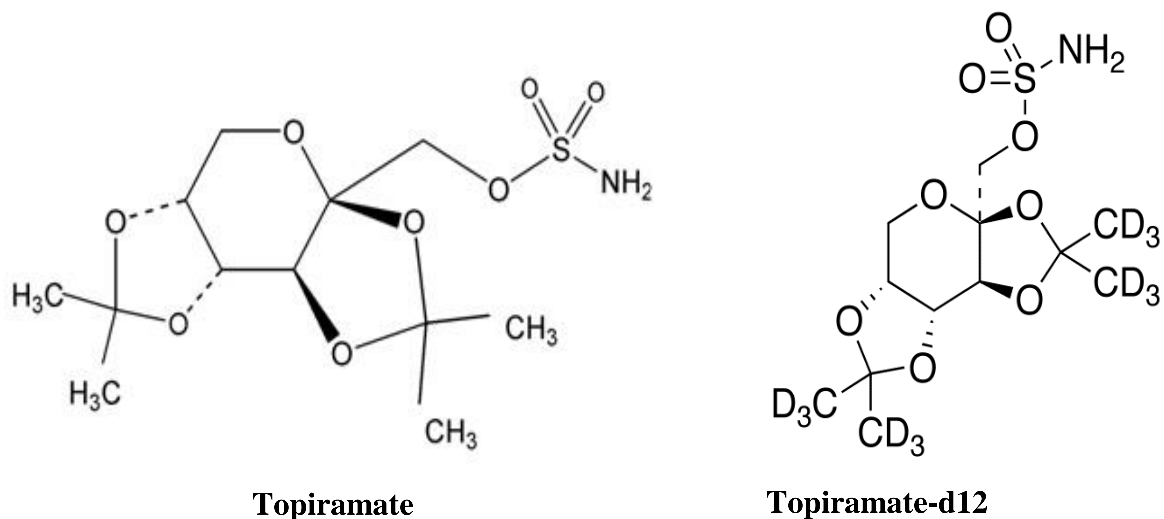
Several LC-MS assays measuring TPM concentrations in plasma have been published.[231,239,240] These methods use plasma volumes of 200-500 µL, which requires sample blood volumes between 0.5 mL and 2 mL. There have been three methods reported that use DBS as the biological sample to measure TPM with LC-MS.[241-243]

Evaluation of the relationship between whole blood and DBS with plasma has been reported.[93,243] Shank and colleagues comparatively assessed TPM PK in 27 healthy male subjects, based on plasma and whole blood data. They found a lower oral clearance, apparent volume of distribution, and longer half-life in whole blood compared to plasma.[93] The difference between TPM whole blood and plasma PK was more profound at low doses (≤100 mg/day). Walker and colleagues completed a correlation study to evaluate the feasibility of DBS collection in anticonvulsants.[243] They did not report any results for topiramate.[243] The assays developed as a part of this project use similar extraction methods to the previous reported assays, however, smaller sample volumes (100 µL) for plasma and whole blood assays and a smaller punch size (3 mm) for the DBS assay were developed to improve upon them.

3.5 EXPERIMENTAL

3.5.1 Chemicals

TPM and TPM-d12 (internal standard) were purchased from Toronto Chemical Research (Ontario, Canada), the chemical structure shown in Figure 4. Methanol and ammonium acetate were of HPLC grade and obtained from Fisher Scientific (MA, USA). Mobile phase was a 20 mM ammonium acetate buffer made by dissolving 1.54 gm of ammonium acetate in 1 L of HPLC grade water. The 1 L buffer was combined with 1 L of methanol. The buffer was filtered with 0.45 micron Nylon filters and degassed prior to use. Perkin Elmer 226 Sample Collection Device cards were used for DBS analysis. Perkin Elmer cards were chosen over other DBS cards because of the cost.



Topiramate **Topiramate-d12**

Figure 4: Chemical Structures of TPM and TPM-d12

3.5.2 Equipment

Instrument Conditions

HPLC analysis was carried out on an Agilent 1100 LC MSD Model G1946 Mass Spectrometer. Data were generated using Agilent ChemStation software. The separation was performed using a Zorbex Eclipse XDB C18 column. The mobile phase consisted of ammonium acetate buffer and methanol (50:50, v/v). The column temperature was maintained at 25°C. The mass spectrometer ionization mode was electrospray with capillary voltage of 4000v and gas temperature of 300°C. Total analysis run time was 10 minutes.

Assays

An analytical method was developed based on previous literature and laboratory experience and validated according the Federal Food and Drug Administration guidance for whole blood, plasma, and DBS.[92]

Preparation of Standard Solutions

TPM-d12 was used as an internal standard (IS), a stock solution of the IS was prepared by dissolving 2.5 mg in 25 ml of methanol to produce a 20 mcg/ml working solution. Stock solutions of TPM were prepared at a concentration of 1 mg/ml in methanol. The stock solutions were further diluted with methanol for the TPM working standard solution. All the solutions were stored at -20 °C.

Calibration standards were freshly prepared on a daily basis by spiking drug-free human whole blood or plasma with appropriate TPM working solutions, in order to produce seven TPM concentrations (0.6-30 mcg/ml).

Preparation of Quality Control Samples

A quality control (QC) stock solution of TPM was prepared at a concentration of 1 mg/ml in methanol by weighing 10 mg into a 10 ml volumetric flask and diluting with methanol. The stock solution was then stored in 2 dram vials at -20°C. The QC stock solution was used to prepare QC samples at three TPM concentration levels: low, medium, and high.

Whole blood QC pools (25 ml) were made by fortifying blank whole blood with 100, 300, and 625 μL of QC solution volume to yield final concentrations of 4, 12, and 25 mcg/ml. Similarly, QC plasma pools (25 ml) were made by fortifying blank plasma with 125, 375, and 625 μL of QC solution volume to yield final concentrations of 5, 15, and 25 mcg/ml. These QC samples were stored in polypropylene tubes at -20°C. QC

samples for DBS were made by fortifying blank whole blood (5 ml) with 22.5, 75, and 135 μL of QC solution volume to yield final concentrations of 4.5, 15, and 27 mcg/ml. Aliquots (30 μL) of QC samples were spotted onto Perkin Elmer 226 DBS cards and allowed to dry at room temperature for at least 3 hours. The dried cards were stored in barrier foil ziplock bags (Ted Pella, Redding, CA) at -20°C. QCs were analyzed during assay validation and with daily standard curves during sample analysis.

Sample Preparation

Ten microliters of each of the working calibration standards were added to 100 μL human whole blood or plasma aliquots to yield final concentrations of the calibration standards in the concentration range of 0.6-30 mcg/mL. Ten microliters of IS were added to each tube and vortexed. Two milliliters of methanol were added to each tube and vortexed for 30 seconds. The sample was then centrifuged for 10 minutes at 1500 rpm at 4°C. The organic layer was transferred into a clean test tube. The solution was evaporated under a stream of nitrogen at 37°C for about 40 minutes using Zymark TurboVap® nitrogen evaporator workstation. The eluate was reconstituted with 200 μL of mobile phase. The extract was transferred into an auto sampler vial and 10 μL was injected into the HPLC column.

Similarly, ten microliters of each of the working calibration standards were added to 50 μL human whole blood to yield final concentrations of the calibration standards in the concentration range of 0.6-30 mcg/mL. Thirty microliters of the prepared spiked blood standards were spotted onto individual Perkin Elmer 226 cards (Perkin Elmer, Greenville,

SC), dried at room temperature for at least 3 hours and stored within a moisture-proof ziplock bag at -20°C until required for analyses. For each DBS, a 3 mm diameter disk (Disposable Biopsy Punch with Plunger, Miltex, PA, USA) was punched manually and placed in an Eppendorf disposable tube. Ten microliters of IS and 500 µL of methanol were added to the disc and the tube was vortex/mixed for 5 minutes. The sample was then centrifuged for 5 minutes at 1000 rpm at 4°C. The organic layer was transferred into a clean test tube. The solution was evaporated under a stream of nitrogen at 37°C for about 20 minutes using Zymark TurboVap® nitrogen evaporator workstation. The eluate was reconstituted with 200 µL of mobile phase. The extract was transferred into an auto sampler vial and 10 µL was injected into the HPLC column.

Red Blood Cell-to-Plasma Partitioning Coefficient

The RBC-to-plasma partitioning coefficient ($K_{RBC:Plasma}$) characterizes the distribution of a compound between RBCs and plasma. The RBC-to-plasma partitioning was established ex vivo using fresh human whole blood with a hematocrit value of 0.36, determined using URIT-12 Hemoglobin Meter (URIT Medical Electronic Co, China). The RBC-to-plasma partitioning coefficient was determined by two methods that have been reported in the literature.[244,245]

In the method developed by Yu et al., an aliquot of 1 ml of human whole blood was pipetted into an Eppendorf polypropylene tube. Twenty, 80, and 100 µL of TPM spiking solution was added so that the final concentrations were 4, 16, and 20 mcg/ml. In parallel, fresh human plasma, prepared from the same batch of fresh whole blood, was

spiked with 20, 80, and 100 μL of TPM to obtain final concentrations of 4, 16, and 20 mcg/ml. The whole blood and plasma sample tubes were gently mixed. The samples were then incubated at 37°C for 1 hour.

For method comparison, TPM was also assayed using the traditional reported method.[245] TPM was added to whole blood to give a final concentration of 5, 10, and 20 mcg/ml. The samples were then incubated at 37°C for 1 hour. The collected plasma or whole blood samples were extracted using a liquid-liquid extraction (described above). The samples were run with seven calibration standards between 0.5-30 mcg/ml.

3.6 RESULTS

3.6.1 Assay Validation

Validation of the developed methods was performed to evaluate the following parameters: selectivity, linearity, accuracy and precision, recovery, and stability. Validation followed the guidelines of the Federal Food and Drug Administration.[216]

Selectivity

Selectivity was determined by analyzing the blank samples from six independent sources for each matrix (whole blood, plasma, and DBS). This ensured that the methods can differentiate and quantify the presence of other components in the sample and be selective for TPM. TPM was found to be well resolved (Figure 5).

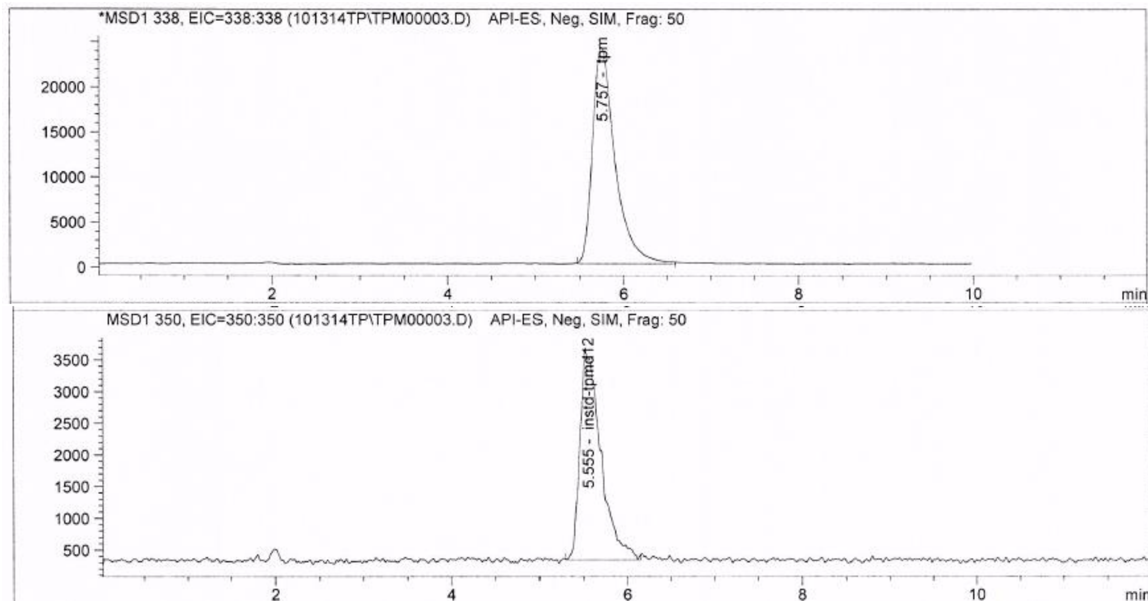


Figure 5: Chromatograph of Topiramate and Internal Standard. Representative LC-MS chromatograms for topiramate (upper trace) and the internal standard (lower trace) in the human blood spot samples.

Linearity

A calibration curve is a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentrations. Five calibration curves were prepared to determine the linearity and variability of the developed assay over a seven concentration range (0.5-30 mcg/ml) (Table 14) of TPM spiked in whole blood, plasma, and DBS samples. Each run also consisted of a blank and a zero sample (blank matrix with IS added). Calibration plots were constructed for peak area ratio (analyte response/IS response) versus the nominal analyte concentration in order to assess the relationship between the two parameters. A weighted linear regression analysis was performed to determine the slope, intercept, and correlation coefficient of the calibration lines.

Table 15: Final TPM concentrations of calibration standards and quality control samples prepared (mcg/ml)

Assay	C1	C2	C3	C4	C5	C6	C7	LQC	MQC	HQC
Whole Blood	0.6	1.2	3	6	12	24	30	4	12	25
Plasma	0.6	1.2	3	6	12	24	30	5	15	25
DBS	0.6	1.2	3	6	12	24	30	4.5	15	27

Several calibration curves were explored using GraphPad Prism™. These included the 1/X and $1/X^2$ weighted linear regression models. Determination of the best fit was based on accuracy and precision. Evaluation of the best fit for each assay indicated that the $1/X^2$ was optimal for whole blood while the 1/X was optimal for plasma and DBS. The calibration curves were found to be linear over the tested concentration range. The correlation coefficient (r^2) was 0.984 or better for all assays. The mean correlation coefficient, slope, and intercept values from the five calibration curves for each assay are presented in Table 16. The average curve of the five calibration curves are shown in Figures 6-8. The %CV at each concentration level for whole blood was $\leq$4.8%, with accuracy ranging from 91.5-95.9%. The %CV at each concentration level for plasma was $\leq$4.5%, with accuracy 94.4-98.9%. The %CV at each concentration level for DBS was $\leq$ 6.5% with accuracy 101.5-106.7%.

Table 16: Mean slope, intercept and correlation coefficient according to the calibration curves plotted (n=5)

Assay	Mean Slope $\pm$ SD	Mean Intercept $\pm$ SD	Mean Correlation Coefficient (r^2) $\pm$ SD
Whole Blood	0.624 $\pm$ 0.042	-0.099 $\pm$ 0.028	0.991 $\pm$ 0.005
Plasma	0.630 $\pm$ 0.015	-0.119 $\pm$ 0.031	0.993 $\pm$ 0.003
DBS	0.136 $\pm$ 0.011	-0.033 $\pm$ 0.012	0.991 $\pm$ 0.002

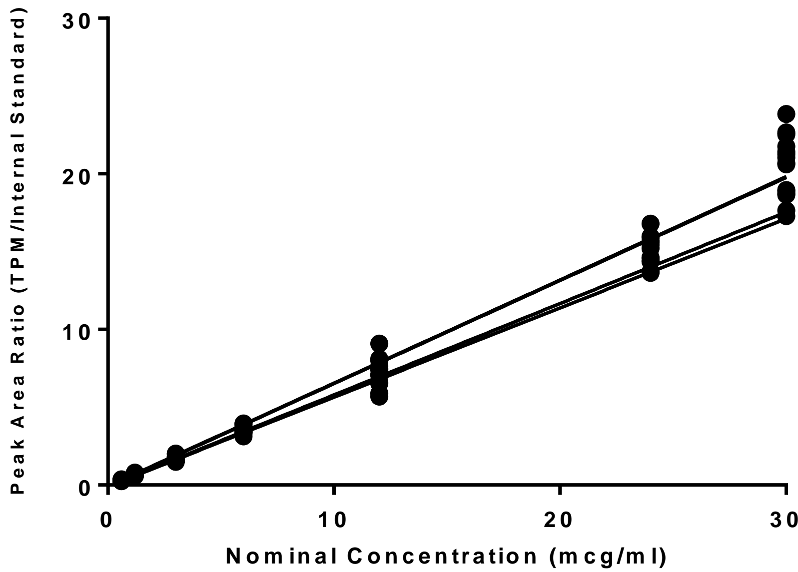


Figure 6: The area response ratio against concentration of TPM in whole blood samples. The response of the instrument with regard to the concentrations of TPM in whole blood samples over 5 days.

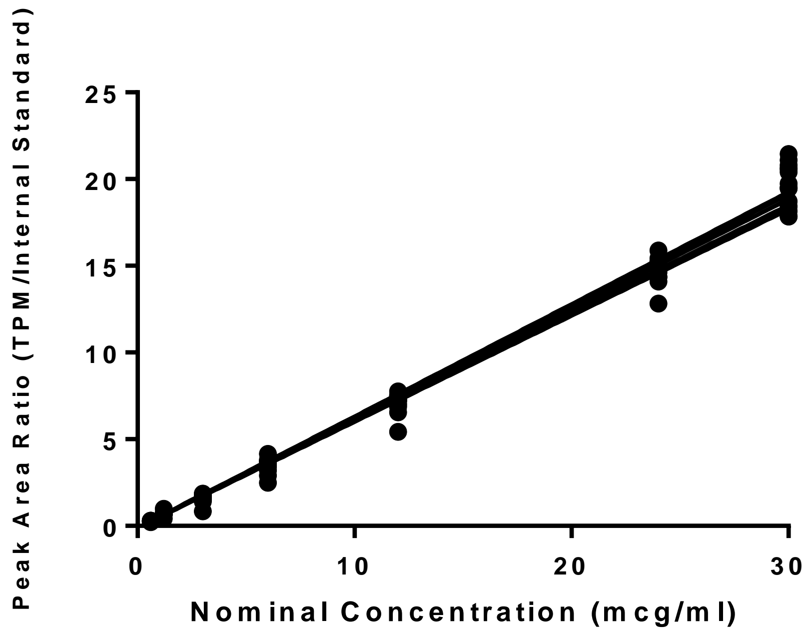


Figure 7: The area response ratio against concentration of TPM in plasma samples. The response of the instrument with regard to the concentrations of TPM in plasma samples over 5 days.

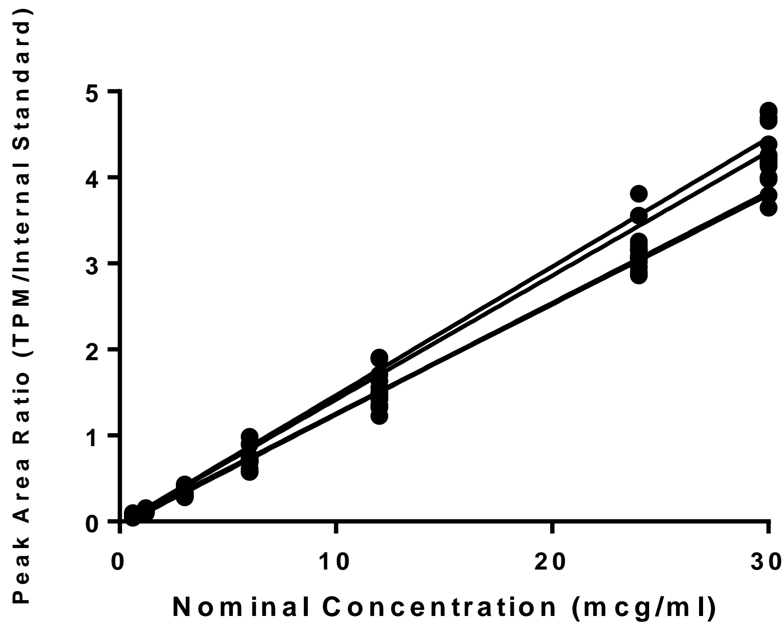


Figure 8: The area response ratio against concentration of TPM in DBS samples. The response of the instrument with regard to the concentrations of TPM in DBS samples over 5 days.

Accuracy and Precision

Within and between day accuracy and precision data were determined for quality control samples for each assay during the 5-day validation (Table 17). In addition to accuracy and precision, there was no statistical difference between days for each assay based on parametric (ANOVA) analysis (p-values > 0.53) (Tables 18-20).

Table 17: Summary of inter-day accuracy and precision for each assay

Assay	Nominal Conc. (mcg/ml)		Intra-day		Inter-day	
			Accuracy (%)	Precision (%CV)	Accuracy (%)	Precision (%CV)
WB	LQC	4	88.5-93.1	1.1-5.1	91.5	3.4
	MQC	12	92.2-98.7	1.8-9.4	95.9	4.8
	HQC	25	88.9-95.7	1.7-8.4	92.8	3.9
Plasma	LQC	5	92.4-100.5	0.6-5.6	95.4	4.5
	MQC	15	91.5-99.1	0.9-4.5	94.4	4.4
	HQC	25	92.4-99.1	1.8-6.1	98.9	4.5
DBS	LQC	4.5	89.7-106	1.8-5	101.5	5.8
	MQC	15	97.2-113.97	0.5-8.3	106.7	6.3
	HQC	27	99.6-114.57	0.8-9.3	106.5	6.5

Table 18: Mean concentration per day of quality control samples for plasma assay

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
LQC	5.0 (0.03)	4.7 (0.27)	4.8 (0.09)	4.7 (0.15)	4.6 (0.20)
MQC	14.9 (0.57)	13.9 (0.44)	14.1 (1.32)	13.8 (0.30)	13.7 (0.12)
HQC	24.8 (0.86)	23.1 (0.41)	23.7 (1.45)	23.7 (1.36)	23.4 (0.47)

Table 19: Mean concentration per day of quality control samples for whole blood assay

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
LQC	3.7 (0.07)	3.6 (0.04)	3.7 (0.19)	3.7 (0.11)	3.5 (0.06)
MQC	11.9 (0.51)	11.4 (0.20)	11.6 (0.51)	11.3 (1.07)	11.1 (0.47)
HQC	23.9 (0.61)	23.1 (0.40)	23.3 (0.49)	22.9 (1.93)	22.2 (0.56)

Table 20: Mean concentration per day of quality control samples for DBS assay

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
LQC	4.6 (0.09)	4.4 (0.08)	4.7 (0.19)	4.7(0.23)	4.8 (0.19)	4.6 (0.22)	4.5 (0.19)
MQC	15.1(0.61)	16 (0.37)	16.8 (0.47)	17.1 (0.09)	15.9 (1.33)	16.6 (0.48)	16.5 (0.46)
HQC	26.89 (1.86)	29.14 (0.56)	30.94 (0.26)	28.76 (0.74)	28.14 (2.60)	30.49 (0.62)	28.53 (0.95)

Percent Recovery

Percent recovery of each assay was determined by comparing the detector response obtained from a known amount of analyte added to, and extracted from, the biological matrix with the detector response obtained from the true concentration of the unextracted standard representing 100% recovery. The calculated recoveries for the whole blood, plasma, and DBS were 80%, 98%, and 96%, respectively.

Limit of Detection (LOD) and Limit of Quantification (LLOQ)

The LOD and LLOQ were calculated using the following formula[246]:

$$LOD = \frac{3.3\sigma}{S} \quad LLOQ = \frac{10\sigma}{S}$$

where σ is the standard deviation of the response, S is the slope of the calibration curve. The results for the LOD and LLOQ for each assay are shown in Table 21.

Table 21: LOD and LLOQ for each assay

Assay	LOD (mcg/ml)	LLOQ (mcg/ml)
WB	0.15	0.45
P	0.16	0.49
DBS	0.39	1.18

Red Blood Cell-to-Plasma Partitioning Coefficient

Two methods were used to measure RBC partitioning of topiramate.[244,245] Both methods yielded comparable results (Figure 9 and 10). The mean at each concentration level are shown in Table 21 and 22. RBC partitioning of a compound may be concentration-dependent if the partitioning involves not only passive diffusion, but also protein binding, RBC components binding or active transporters.[244] As indicated in Figure 9 and 10, the

partitioning of TPM was concentration-dependent in the concentration range of 1 – 20 mcg/ml.

Table 22: Mean values of concentration based on new assay method

Nominal Concentration (mcg/ml)	Reference Plasma Concentration (mcg/ml) (Mean ± SD)	Equilibrated Plasma Concentration (mcg/ml) (Mean ± SD)	Calculated $K_{RBC/P}$ (Mean ± SD)
0.8 (N=3)	0.78 ± 0.03	0.22 ± 0.01	22.89 ± 3.40
4 (N=8)	4.49 ± 0.45	1.33 ± 0.69	8.45 ± 4.04
16 (N=8)	17.36 ± 6.43	13.48 ± 6.12	3.96 ± 0.33
20 (N=9)	22.61 ± 6.49	16.46 ± 5.99	1.94 ± 0.07

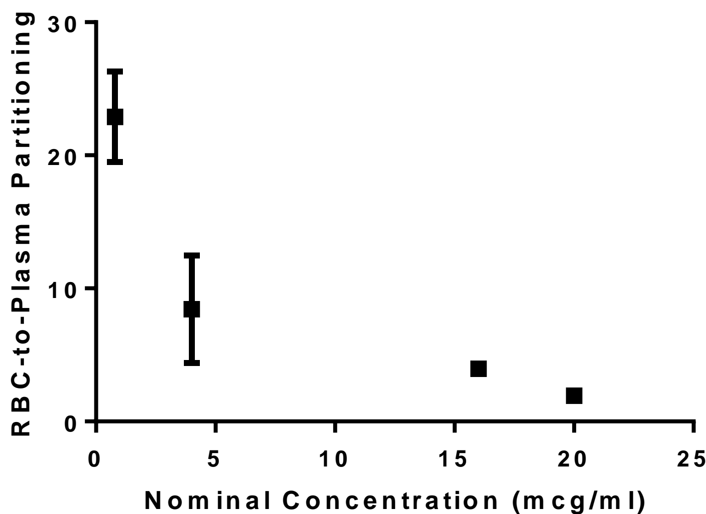


Figure 9: Effect of topiramate concentration on RBC partitioning using reference plasma and equilibrated plasma. Effect of compound concentration on RBC partitioning (incubation time: 60 minutes) (N=28).

Table 23: Mean values of concentrations based on traditional assay method

Nominal Concentration (mcg/ml)	Spiked Conc. Whole Blood (mcg/ml) (Mean + SD)	Analyzed Conc. Plasma (mcg/ml) (Mean + SD)	Blood-to-Plasma Ratio	Calculated Conc. RBC (mcg/ml)	RBC-to-Plasma Partitioning
5 (N=4)	5.25 + 0.12	2.87 + 0.30	1.85 + 0.18	9.19 + 0.29	3.22 + 0.38
10 (N=4)	9.89 + 0.35	7.22 + 0.12	1.38 + 0.06	14.27 + 1.08	1.99 + 0.12
20 (N=3)	19.66 + 0.65	17.33 + 0.29	1.14 + 0.05	23.47 + 2.04	1.36 + 0.14

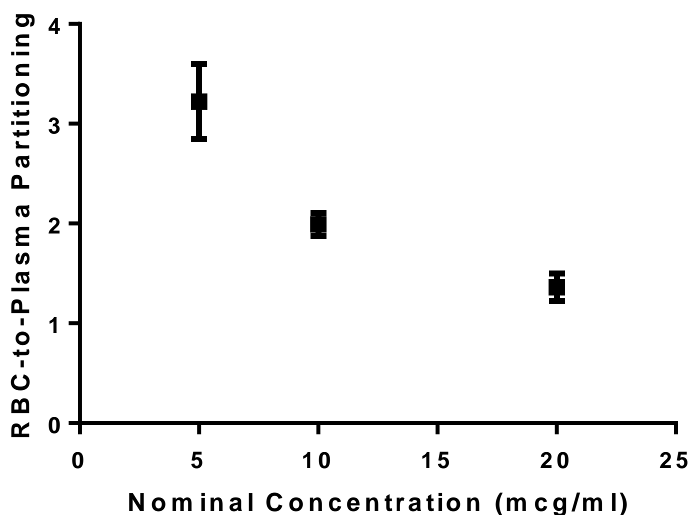


Figure 10: Effect of topiramate concentration on RBC partitioning using whole blood and plasma. Effect of compound concentration on RBC partitioning (incubation time: 60 minutes) (N=11).

3.7 CONCLUSION

The method developed for the quantification of TPM in whole blood, DBS, and plasma was successfully validated according to FDA guidelines. Perkin Elmer cards were shown to be suitable for this application. We demonstrated that the difference in extraction recovery between assays is small suggesting topiramate can be measured in a whole blood, plasma, and DBS. Successful validation confirmed that these methods are suitable

for the determination of TPM in whole blood, plasma, and DBS samples in the following studies and will be used to determine TPM concentratin in canine and human samples.

CHAPTER 4: WHOLE BLOOD TPM PHARMACOKINETICS FOR INTRAVENOUS AND ORAL DOSING IN DOGS WITH NATURALLY-OCCURRING EPILEPSY

4.1 INTRODUCTION

An intravenous formulation could be beneficial for use in adults and animals; however, low drug solubility hindered its development. Researchers at the Center for Orphan Drug Research (CODR) overcame this challenge by using a cyclodextrin excipient to increase solubility. Based on this work, a novel IV TPM formulation, 10 mg/ml dissolved in 10% sulfobutyl cyclodextrin (Captisol®), was developed by the CODR at the University of Minnesota which has been licensed to CureX. Cyclodextrins are complex molecules that have a hydrophilic exterior and a lipophilic interior cavity. These molecules can improve drug solubility and stability by attracting poorly water soluble compounds into the cavity while the cyclodextrin-drug combination remains in solution. Drug rapidly dissociates from the cyclodextrin once the solution is injected into the bloodstream.[247,248] Captisol® (Cydex) is a polyanionic β-cyclodextrin derivative that has an improved safety profile over other the parent β-cyclodextrin by eliminating the potentially damaging effects. It has been shown to be safe in humans and has received FDA approval for use in parental formulations of voriconazole (VFEND®), aripiprazole (Abilify®), and ziprasidone (GEODON®).

Two studies have been completed in adult patients (n=20) and healthy volunteers (n=12) that established the PK and safety of the IV TPM.[91,92,249] The first study reported the PK parameters and safety of IV TPM in patients with epilepsy or migraines. The second was a two-way crossover study of oral and IV TPM in healthy volunteers designed to determine safety, estimate PK parameters, and determine oral bioavailability. The results

of these two studies show the absolute bioavailability of oral TPM is approximately 100%.

Canine epilepsy closely resembles human epilepsy and is being utilized by our research group to study approved and investigational antiepileptic drugs. An ongoing study of TPM in these dogs allowed further exploration of the nonlinear relationship between TPM plasma and whole blood concentrations.

The objective of this study was to characterize the PK of IV and oral TPM in dogs and the relationship between plasma and whole blood concentrations. This is the first animal study to determine whole blood TPM PK and the relationship between whole blood and plasma topiramate concentrations.

4.2 STUDY AIMS

The primary objectives of this study were to compare the PK of IV TPM in plasma and whole blood and describe the relationship between whole blood and plasma concentrations in dogs with naturally occurring epilepsy.

Aim #1: Characterize the relationship between whole blood to plasma TPM concentrations in dogs.

Aim #2: Characterize and compare the TPM pharmacokinetics measured in whole blood and plasma.

4.3 STUDY METHODS

4.3.1 Animals and Study Design

Animal experiments were conducted in accordance with the Animal Welfare Act for the care and use of laboratory animals. The project was approved by the University of Minnesota Institutional Animal Care and Use Committee. The experiment was carried out in four dogs with naturally occurring epilepsy. Dogs were housed at the Veterinary Center at University of Minnesota in St. Paul, Minnesota. Animals were fasted overnight prior to each dose and were 2 hours post-injection.

Each dog was administered a single dose of 10 mg/kg stable-isotope ($^{13}[C]_6$) topiramate via a bolus injection (over 5 minutes) and 5 mg/kg non-labeled topiramate orally 1 hour post-injection. The oral TPM was given 1 hour post-injection so it would not produce confounding factors when recording the response of the IV TPM on iEEG recordings (data not presented in this thesis). A second dose of 20 mg/kg was administered to three additional dogs. Whole blood samples (~2 ml) were collected via catheterized vein pre-dose and at 0, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 9, and 24 hours post-injection (Table 24). A 250 µL sample of whole blood was aliquoted, the remaining blood was placed on ice, plasma separated, and immediately frozen (-20°C) until analysis. Missed samples were recorded.

Table 24: Blood Collection Times (in minutes)

IV	predose	0	5	15	30	45	60	90	120	150	180	240	360	480	540
ORAL							0	30	60	90	120	180	300	420	480

4.3.2 Study Drug and Dose Rationale

Stable-labeled IV TPM solution was provided by CODR and unlabeled TPM tablets (100 mg, Johnson and Johnson) were purchased by the Veterinary Medical Pharmacy. The pharmaceutical grade unlabeled TPM tablets were purchased from the Pharmacy at the Veterinary Medical Center. The stable-labeled topiramate solution was prepared by the Pharmaceutical Service Division at the University of Iowa under good manufacturing procedures. The solution is 10 mg/ml topiramate in 10% Captisol®.[249] Using intravenous data from one dog in a study by Streeter et al, we estimated an apparent volume of distribution of 0.63 L/kg.[250] Thus, in a healthy beagle dog, a 10 mg/kg intravenous dose of topiramate was estimated to produce an initial concentration (C_0) of 15.9 mcg/ml.

4.3.3 Drug Assay

Whole blood topiramate concentrations were quantified using the LC-MS method described in Chapter 3.

4.3.4 TPM Binding to Carbonic Anhydrase

TPM dissociation binding constant (K_D) and maximum binding capacity (B_{max}) values for its saturable binding to erythrocytes was obtained by applying equation 1 that contained either 1 or 2 binding sites to the data.

$$C_B = \frac{B_{max} * C_P}{C_P + K_D} + \frac{B_{max2} * C_p}{C_p + K_{D2}} \tag{1}$$

In equation 1, C_B represents the bound concentration of TPM and C_p represents the total concentration in plasma. Kinetic constants were obtained using a non-linear regression analysis to fit the data, assuming either 1 or 2 saturable binding sites (Phoenix software, Version 6.3; Pharsight Corporation, Mountain View, CA, USA). The results of the one-site model versus the two-site model were compared for statistical significance using an F-test.

4.3.5 Pharmacokinetic Analysis Methods

Non-compartmental analysis

Concentration-time data from the intravenous and oral administrations were analyzed to obtain area under the curve (AUC), clearance, half-life, volume of distribution, and bioavailability using a non-compartmental PK approach with Phoenix software (WinNonLin, Version 6.3; Pharsight Corporation, Mountain View, CA, USA). All data was weighed using uniform weighing. To determine the absolute bioavailability, the areas under the concentration-time curve for both the oral and intravenous topiramate were calculated. The $AUC_{0\text{-}\infty}$ was calculated using a log-linear trapezoidal method with the tail area calculated from C_{last}/k. The terminal rate constant (k) was determined by linear regression of the terminal phase on log concentration versus time plots. Terminal half-life ($T_{1/2}$) was calculated as 0.693/k.

The maximum concentration (C_{max}) was the highest observed whole blood oral TPM concentration. Clearance was estimated by dose divided by $AUC_{0-\infty}$. Volume of distribution was estimated by dose divided by k*$AUC_{0-\infty}$.

Bioavailability (F) for each dog and mean bioavailability was determined by calculating the ratio of the dose normalized oral area under the concentration-time curves ($AUC_{0-\infty}$) to the dose normalized intravenous area under the concentration-time curves ($AUC_{0-\infty}$) (Equation 2).

$$F = \frac{Dose_{IV} \ x \ AUC_{Oral}}{Dose_{Oral} \ x \ AUC_{IV}} \tag{2}$$

Compartmental analysis

Concentration-time data were analyzed using compartmental methods (WinNonLin, Version 6.3; Pharsight Corporation, Mountain View, CA, USA). One- and two-compartmental models were evaluated. Data were analyzed using different weighting schemes ($1/y$, $1/y^2$, $1/\hat{y}$, $1/\hat{y}^2$). Models were compared using visual inspection, goodness of fit plots, weighted residual plots, precision of model parameters, weighted residual sum of squares, and Akaike's information criterion.

4.4 RESULTS

A total of 7 dogs were used in this experiment. Four dogs received an intravenous bolus TPM dose of 10 mg/kg and an oral topiramate dose of 5 mg/kg 60 minutes after the IV

dose. An additional three dogs received an intravenous bolus TPM dose of 20 mg/kg. The dogs weighed between 15-34 kilograms at the time of dosing. Three dogs were on comedications (PB, PB and LEV, or PB and LEV, and zonisamide (ZNS)). There were a total of 51 missing blood samples out of 98 total samples for all dogs due to limited amount of sample or the blood sample was not separated prior to centrifuging.

The individual concentration-time profiles of labeled and unlabeled TPM are shown in Figure 11-13, respectively. The graphs show two distinct groups, dogs which were on enzyme-inducing medication and dogs which were not on any medications. Concentrations were substantially lower in the inducer group. Concentrations fall below 10 mcg/ml at 30 minutes for inducers whereas concentrations didn't fall below 10 mcg/ml until 480 minutes for non-inducers. Figure 13 shows that the T_{max} ranged from 1-2 hours in all dogs and C_{max} ranged from 2-8 mcg/ml.

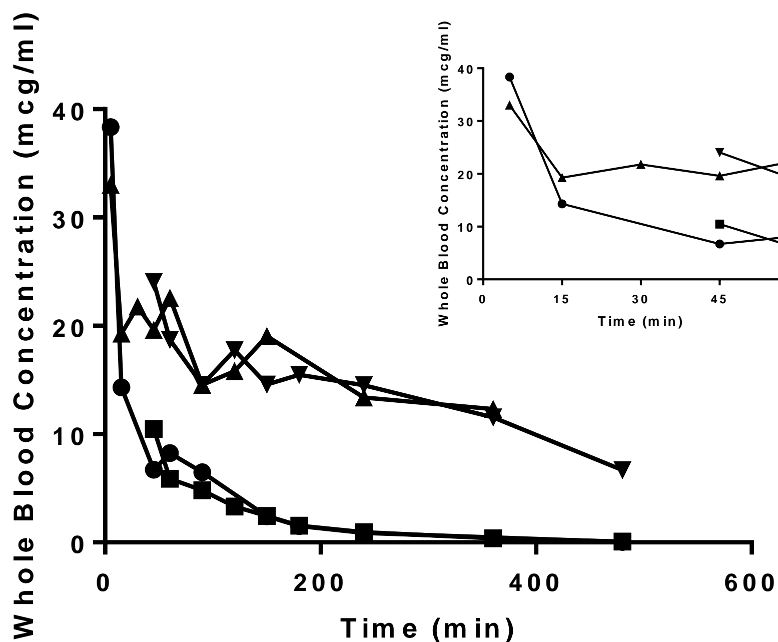


Figure 11: Individual whole blood concentration-time profile for 10 mg/kg labeled IV topiramate. The symbols represent each dog. Circles: dog 1, squares: dog 2, triangles: dog 3, and inverted triangles: dog 4.

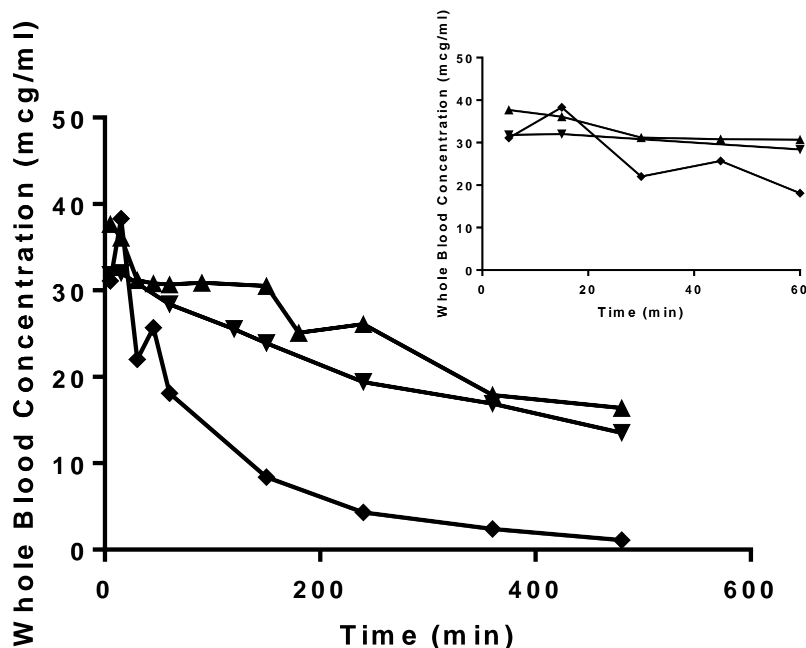


Figure 12: Individual whole blood concentration time profile for 20 mg/kg labeled IV TPM. The symbols represent each dog. Triangles: dog 3, inverted triangles: dog 4, and diamond: dog 5.

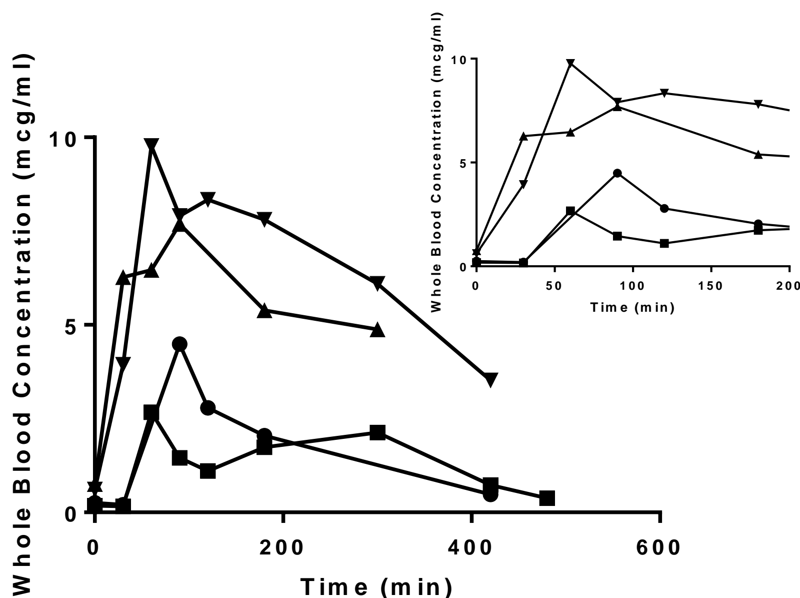


Figure 13: Individual whole blood concentration-time profile for unlabeled oral topiramate. The symbols represent each dog. Circles: dog 1, squares: dog 2, triangles: dog 3, and inverted triangles: dog 4.

4.4.1 Non-compartmental analysis

Table 25 summarizes the individual PK parameters for all dogs. The clearance of topiramate was 0.3 and 0.6 L/hr/kg in dogs on inducing comedication and 0.04 and 0.07 L/hr/kg in dogs not on inducing comedication. The dogs with the highest clearance were on PB, an inducing AED. The volume of distribution was 0.06 to 0.68 L/kg in inducers and 0.23 and 0.4 in non-inducers. In dogs on PB, the half-life was 0.9 to 1.7 hours. In dogs not on additional medications, the half-life was 4.2 and 4.6 hours. The half-life increased to 6.4 and 7.0 hours for non-inducers on the higher dose. The mean absolute bioavailability for orally administered topiramate was 87% $\pm$ 40% with a range of 57% to 144%. The maximum concentration of the stable-labeled topiramate after 10 mg/kg and 20 mg/kg given intravenously was 38.4 mcg/ml and 37.7 mcg/ml and was not affected by whether they were on enzyme-inducing medication.

Table 25: Individual pharmacokinetic parameters

ID	Dose (mg/kg)	CL (L/hr/kg)	V_d (L/kg)	$T_{1/2}$ (hr)	$C_{max,oral}$ (mcg/ml)	$AUC_{0-\infty,iv}$ (mcg*hr/ml)	$AUC_{0-\infty,oral}$ (mcg*hr/ml)	F (%)	Inducing Comed
1	10	0.05	0.06	0.9	4.5	203.4	12.3	62	Yes
2	10	0.30	0.42	1.0	2.7	33.11	11.5	144	Yes
3	10	0.04	0.23	4.6	7.7	236.1	67.1	57	No
4	10	0.06	0.37	4.2	9.8	145.1	61.8	85	No
3	20	0.06	0.56	7.0	NC	359.9	NC	NC	No
4	20	0.07	0.63	6.4	NC	291.0	NC	NC	No
5	20	0.30	0.68	1.7	NC	67.3	NC	NC	Yes

NC: not calculated

4.4.2 Compartmental analysis

One and two-compartment intravenous infusion models were evaluated using the whole blood TPM concentrations. A one-compartment model best fit the data. Goodness of fit plots, including weighted residuals versus time and observed versus predicted values are shown in Figure 14 through Figure 15. While the residual plot suggested a two-compartment model may be better, the parameter estimates were unstable with poor precision (high CVs). This is likely because of the small sample size and lack of samples at the early time points. A weighting scheme of $1/\hat{y}$ was used for the intravenous 1-compartment model and $1/\hat{y}^2$ for oral 1-compartment.

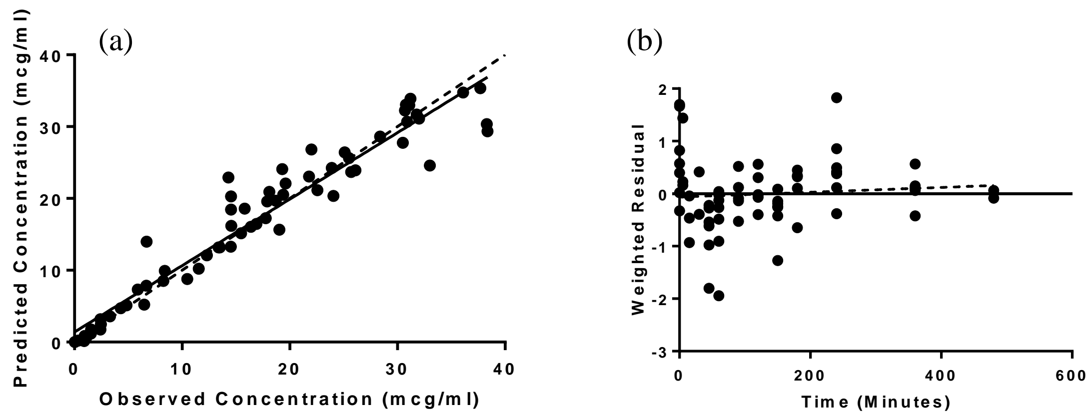


Figure 14: Goodness of fit for one-compartment model after intravenous dose in all dogs. (a) Identity plots of observed vs. individual predicted TPM concentration. (b) Scatter plot of weighted residuals (WRES) vs. time. Line of identity (solid); regression line (dashed)

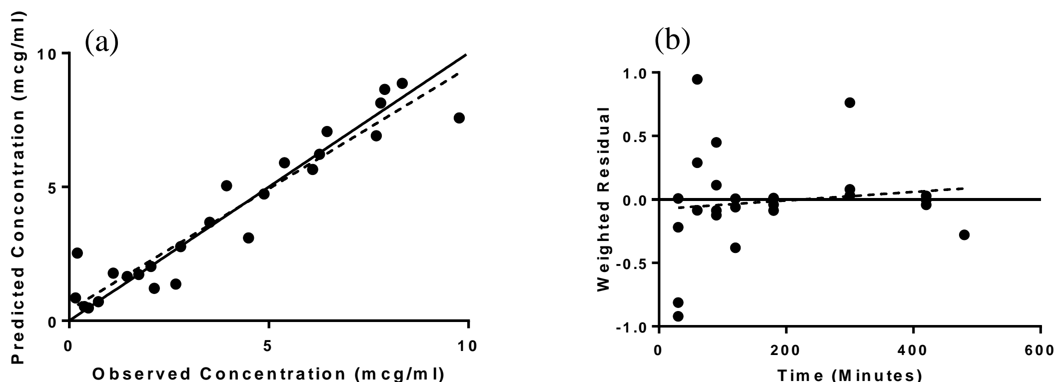


Figure 15: Goodness of fit for one compartment model after oral dose. (a) Identity plots of observed vs. individual predicted TPM concentration. (b) Scatter plot of weighted residuals (WRES) vs. time. Line of identity (solid); regression line (dashed)

The individual PK parameters for the 1-compartment IV and oral TPM model are shown in Table 26 and Table 27. The individual PK parameters from the one-compartment model compared closely to the results from the non-compartmental analysis. The clearance for non-inducers was 0.04 – 0.07 L/hr/kg with the non-compartmental methods compared to 0.03 – 0.1 L/hr/kg with the 1-compartment model. The half-life was 0.9 – 1 and 1 - 1.4 hours with the non-compartmental and 1-compartment models for inducers, respectively. The half-life ranged from 4.2 – 4.6 and 5.1 – 8.3 hours with the non-compartmental and 1-compartment models for non-inducers, respectively.

Table 26: Individual 1-compartment pharmacokinetic parameters after intravenous topiramate

	ID	Dose (mg/kg)	V_d (L/kg)	$T_{1/2}$ (hr)	CL (L/hr/kg)	K_{10} (1/hr)
Inducers						
	1	10	0.2	1.0	0.2	0.9
	2	10	0.7	1.0	0.5	0.7
	5	20	0.6	1.4	0.3	0.5
Non-inducers						
	3	10	0.4	8.1	0.03	0.09
	4	10	0.4	5.3	0.1	0.13
	3	20	0.6	6.9	0.1	0.10
	4	20	0.6	6.3	0.1	0.11

Table 27: Individual 1-compartment pharmacokinetic parameters after oral topiramate

	ID	V_d (L/kg)	$T_{1/2}$ (hr)	CL (L/hr/kg)	K_{10} (1/hr)	C_{max} (mcg/ml)	AUC (mcg*hr/ml)
Inducers							
	1	1.0	1.9	0.4	0.4	3.2	13.3
	2	1.0	1.6	0.4	0.4	1.8	11.2
Non-inducers							
	3	0.6	6.3	0.07	0.1	7.1	72.1
	4	0.4	3.1	0.08	0.2	8.9	61.2

4.4.3 Comparison of Plasma and Whole Blood Pharmacokinetics

Figure 16-19 show individual concentration-time data for whole blood and plasma concentrations after intravenous dosing and oral dosing separated by inducing and non-inducing medications. Concentrations for the non-inducing dogs have a larger difference between whole blood and plasma TPM concentrations.

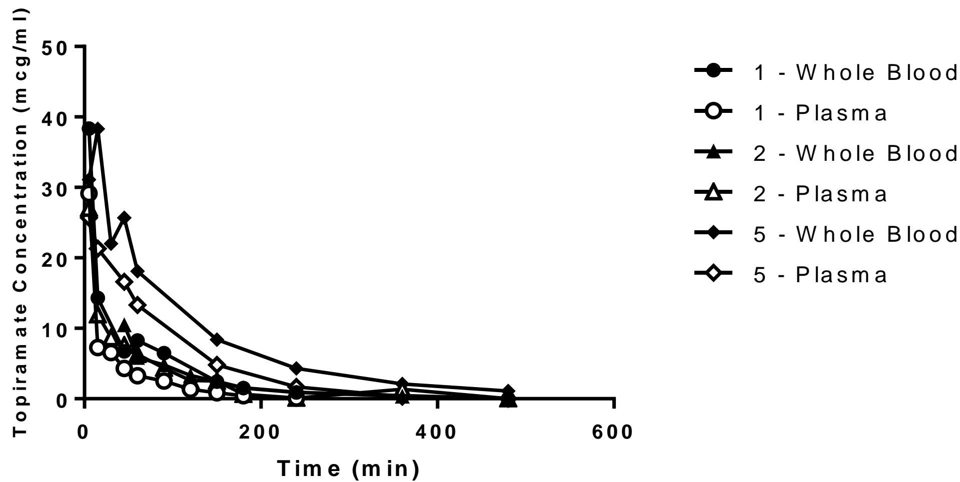


Figure 16: Concentration-time profile after intravenous dosing for inducers. The symbols represent dog 1 (circles), dog 2 (triangles), and dog 5 (diamonds). Open symbol represents plasma concentrations and filled symbol represent whole blood concentrations.

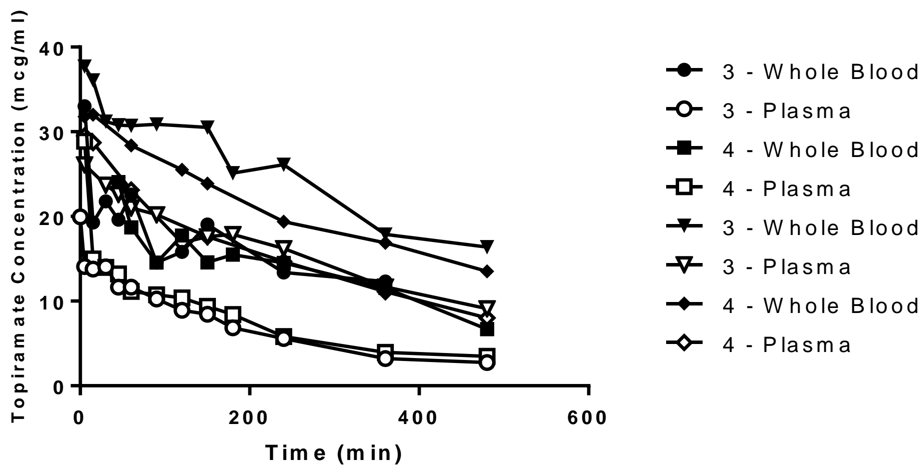


Figure 17: Concentration-time profile after intravenous dosing for non-inducers. The symbols represent dog 3 at 10 mg/kg dose (circles), dog 3 at 20 mg/kg dose (inverted triangles), dog 4 at 10 mg/kg dose (squares) and dog 4 at 20 mg/kg dose (diamonds). Open symbol represents plasma concentrations and filled symbol represent whole blood concentrations.

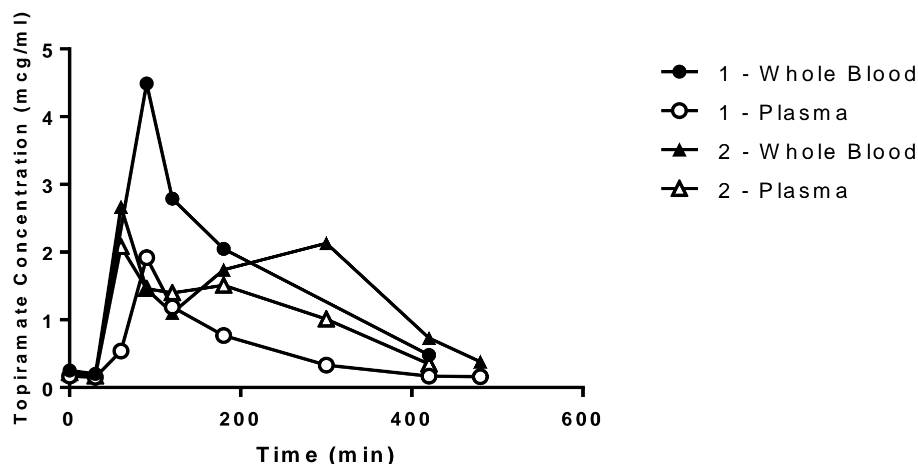


Figure 18: Concentration-time profile after oral dosing for inducers. The symbols represent dog 1 (circles) and dog 2 (triangles). Open symbol represents plasma concentrations and filled symbol represent whole blood concentrations.

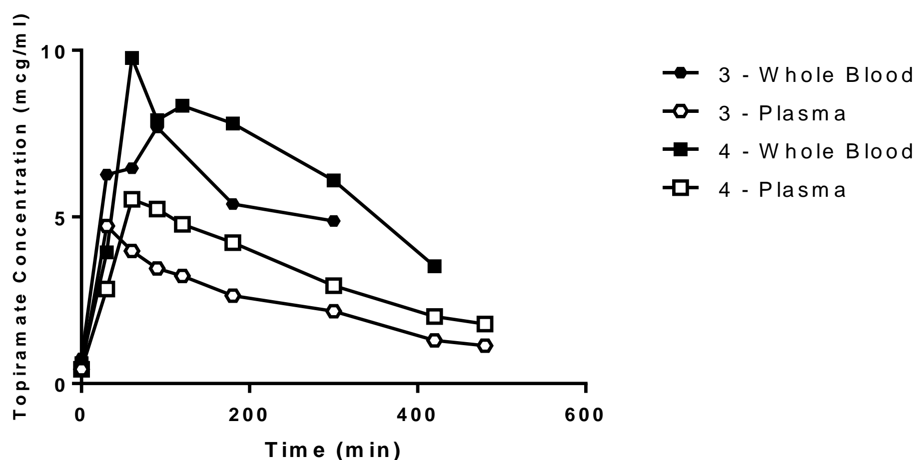


Figure 19: Concentration-time profile after oral dosing for non-inducers. The symbols represent dog 3 (circles) and dog 4 (squares). Open symbol represents plasma concentrations and filled symbol represent whole blood concentrations.

Individual PK parameters for whole blood and plasma are shown in Table 28. While maximum concentrations in whole blood and plasma were similar, AUCs were greater in whole blood resulting in lower clearance. Non-compartmental analysis showed the clearance of TPM ranged from 0.04 to 0.30 L/hr/kg and 0.11 to 0.88 L/hr/kg for whole

blood and plasma, respectively. The volume of distribution varied from 0.06 to 0.68 L/kg and 0.5 to 0.7 L/kg for whole blood and plasma, respectively. The half-life ranged from 0.9 to 4.6 hours and 0.5 to 3.7 hours for whole blood and plasma. Half-life and clearance were affected by inducing comedication use to the same degree for both whole blood and plasma.

Table 28: Individual intravenous non-compartmental pharmacokinetic parameters for whole blood and plasma

	ID	V_d (L/kg)	$T_{1/2}$ (hr)	CL (L/hr/kg)	$AUC_{0-\infty}$ (mcg*hr/ml)
Whole Blood					
	1	0.06	0.9	0.05	203.4
	2	0.42	1.0	0.30	33.1
	3	0.23	4.6	0.04	236.1
	4	0.37	4.2	0.06	145.1
	5	0.68	1.7	0.30	67.3
Plasma					
	1	0.59	0.5	0.88	11.4
	2	0.50	0.7	0.46	21.7
	3	0.66	3.7	0.12	81.7
	4	0.66	3.5	0.11	94.5
	5	0.70	0.9	0.15	38.1

4.4.4 TPM Binding to Carbonic Anhydrase

Whole blood and plasma concentrations were different at lower concentrations. Additionally, the whole blood-to-plasma ratio changed with plasma concentrations being higher at lower plasma concentrations indicating that TPM is distributing into RBCs. The distribution into RBCs is likely due to its binding to carbonic anhydrase isoforms that are present in high concentrations in the erythrocytes.[251] Figure 20 shows the whole blood/plasma concentration ratio for TPM as a function of the plasma concentration

excluding the dog taking ZNS. The figure suggests there is greater binding at lower concentrations that becomes saturated as plasma concentration increases.

Binding analysis revealed that binding comprised a saturable component (Figure 20). When a single binding site model was assumed, the optimal fit yielded a K_D of 1.2 mcg/ml (95% confidence interval, CI = 0.3 – 2.6) and a B_{max} of 2.9 mcg/ml (95% confidence interval, CI = 1.9-3.8). The two saturable binding sites model was unable to fit the data and was over-parameterized given the small sample size.

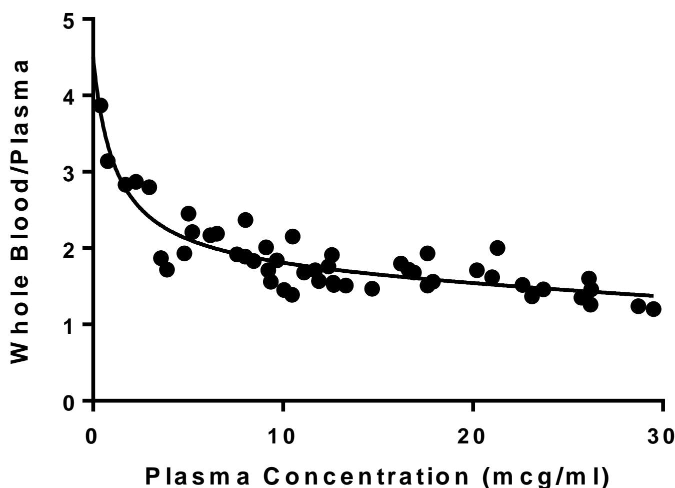


Figure 20: Plot of the whole blood/plasma concentration ratio for TPM as a function of plasma concentration. The curvilinear line was formed by a curve-fit analysis of the data assuming one saturable binding site.

4.5 DISCUSSION

This pilot study examined the whole blood PK of IV and oral TPM in dogs and compared plasma and whole blood TPM concentrations. Similar to humans, there was a nonlinear relationship between plasma and whole blood concentrations in which whole blood TPM

concentrations are 1.3-3.9 times greater than plasma concentrations. This relationship is important when predicting plasma concentration from whole blood and appears to apply to both dogs and humans. Caution should be exercised in interpreting whole blood TPM concentrations as one may overestimate plasma concentrations by as much as 4 fold.

Erythrocytes of dogs also contain CA-I and CA-II where CA-II has higher enzymatic activity which is similar to humans.[251] There was one dog that had lower and more constant plasma to whole blood ratios. Interestingly, this dog was taking ZNS as a concomitant medication. ZNS is structurally similar to topiramate and has also been suggested to bind to the two isoforms of carbonic anhydrase in red blood cells.[252] This may suggest competitive binding between TPM and ZNS. This dog was excluded from the analysis of TPM binding to carbonic anhydrase. The one saturation binding site model had a higher K_D and lower B_{max} than values reported previously in humans.[93] Given the small number of samples collected, the parameters for the two saturable binding site model were not able to be estimated. Previous reports have described a two saturable binding site model which would be expected given the two isoforms of carbonic anhydrase located in the red blood cells.

There was a difference in PK parameter estimates with those derived from whole blood generally showing reduced clearances and volumes resulting in longer elimination half-lives. This study found the mean oral bioavailability was 78% which is greater than the range of 27 to 59% that has been reported for beagle dogs.[250] However, half-life was similar to a previously reported study, 3.4 hours compared to 4.4 hours for non-

inducers.[250] When using TPM in dogs, consideration should be taken if the animals are on known enzyme inducers as they may need higher doses to attain the target concentrations.

While the established PK model did provide useful information it is limited by both the small number of dogs and the limited sample volume in whole blood. The observation of a difference between whole blood and plasma is supported by other work in humans.[93,235] The small sample size resulted in the two compartment model being unstable. Non-compartmental and the one-compartmental analysis resulted in elimination half-life, clearance, and volume of distribution values that agree well with previously published literature.[250]

Assuming the rapid distribution of TPM from plasma to other body compartments includes the central nervous system; IV TPM may be useful in treating canine seizure emergencies and may have potential use in human seizure emergencies.

This work demonstrates that using a small number of dogs can be informative in establishing the PK of an AED therapy and characterizing the binding properties of topiramate to carbonic anhydrase in the red blood cells. Further study is needed on the use of IV TPM for seizure emergencies.

CHAPTER 5: COMPARISON OF TOPIRAMATE CONCENTRATIONS IN WHOLE BLOOD AND PLASMA IN ADULTS AND CHILDREN

5.1 INTRODUCTION

The goal of this research project is to characterize the relationship of whole blood, DBS, and plasma TPM concentrations in adults and children.

DBS technology has a number of advantages over conventional sample collection. These include practical, clinical, and cost considerations. Use of DBS technology allows for less invasive sampling (finger or heel prick rather than a venipuncture) and as much as a 90% reduction blood volume needed to perform the assay. Further, use of DBS can simplify sample collection and make shipping of samples easier. However, as previously discussed (Chapter 3), one problem with using DBS to measure TPM levels is that TPM exhibits saturable binding to carbonic anhydrase in red blood cells.[93] This creates a nonlinear relationship between whole blood and plasma concentrations and results in higher whole blood concentration versus plasma concentration, particularly at TPM concentrations near the lower limit of its reference range (2-20 mg/L).[218,219] Thus, TDM of TPM using whole blood as a matrix may mislead the clinician, as a plasma or serum is the normal matrix where TPM is measured. Further, PK parameters determined using TPM concentrations derived from whole blood may differ from those based on plasma-based assay, resulting in confusion about a drug's disposition.

Few studies have been reported using DBS for TPM. One study used DBS in children between 0.9-16 years to determine adherence of antiepileptic medications.[217] They concluded that DBS was a useful approach to adherence assessment when combined with other measures including self-reporting. Information about TPM concentrations in

children measured with DBS is limited. Two studies have evaluated TPM concentrations in neonates using DBS.[109,110] These studies converted the DBS concentrations into plasma concentrations using a possibly flawed assumption that could result in incorrect estimation of plasma TPM concentrations. There are no published reports that have characterized the relationship of TPM concentrations measured in blood and plasma of children.

5.2 STUDY AIM

The primary objective of this study is to compare TPM concentrations measured in whole blood, DBS, and plasma in samples obtained from adults and children taking TPM for any indication.

The Specific Aim of this project is to:

Aim #1: Characterize the relationship between whole blood or DBS TPM concentrations to plasma TPM concentrations in human samples

5.3 METHODS

5.3.1 Subjects and Study Procedures

Thirty (30) adult patients, 18 years of age and older and eight (8) pediatric patients, ages 5 years to 12 years, were enrolled in the study. Patients were recruited from the MINCEP Clinic of the University of Minnesota Physicians, Gillette Children's Specialty Healthcare, and the Minnesota Epilepsy Group Clinic. The study was approved by the IRB at the University of Minnesota.

Prospective subjects were identified from the participating clinic database, eligibility reviewed by the principal investigator, and potential subjects were presented to the attending physician. The attending physician briefly reviewed the study with the patient during the routine clinic visit. If the subject was interested, the principal investigator further discussed the study with patients at their clinic visit, answered any questions and invited the patient to participate in the study. Informed consent was then presented to potential subjects after their clinic visit. Subjects unwilling to have blood drawn or a finger-prick were excluded.

Two one-milliliter blood samples were collected. One blood sample was used to assay TPM in whole blood and the second was processed for plasma for measurement of TPM concentations. Patients also underwent a finger prick to collect a DBS.

Drug Assay

The analytical method was designed to measure TPM in plasma, whole blood, and DBS using LC-MS. These methods are described in Chapter 3.

5.4 DATA ANALYSIS

5.4.1 TPM Binding to Carbonic Anhydrase

TPM dissociation binding constant (K_D) and maximum binding capacity (B_{max}) values for its saturable binding to erythrocytes was obtained by applying equation 3 that contained either 1 or 2 binding sites to the data.

$$C_B = \frac{B_{max} * C_P}{C_P + K_D} + \frac{B_{max2} * C_p}{C_p + K_{D2}} \quad (3)$$

where C_B represents the bound concentration of TPM and C_p represents the total concentration in plasma. Kinetic constants were obtained using a non-linear regression analysis to fit the data, assuming either 1 or 2 saturable binding sites (Phoenix software, Version 6.3; Pharsight Corporation, Mountain View, CA, USA). The results of the one-site model versus the two-site model were compared for statistical significance (p-value < 0.05) using an F-test.

5.4.2 Estimation of Plasma Concentrations

Whole blood is composed of serum, red blood cells, white blood cells, and platelets; where the fractional volume of white blood cells and platelets is negligible. The plasma-to-whole blood concentration ratio depends on plasma protein binding, partitioning into blood cells, and the volume occupied by blood cells. The following equation can describe this relationship, where C_b is the TPM concentration in whole blood, C_p is the TPM concentration in plasma, V_p is the plasma volume, C_{RBC} is the TPM concentration in red blood cells, V_{RBC} is the volume occupied by the red blood cells, and V_b is the volume of whole blood.

$$C_b * V_b = C_p * V_p + C_{RBC} * V_{RBC} \quad (4)$$

The concentration of analyte in whole blood can then be calculated from the following equation.

$$C_b = \frac{C_p * V_p + C_{RBC} * V_{RBC}}{V_b} \quad (5)$$

For TPM, the whole blood and plasma concentration will be different at low concentrations. The difference depends on the concentration in the red blood cells and the volume of plasma and red blood cells in whole blood. The concentration of the analyte in red blood cells relies upon the red blood cell-to-plasma partitioning ($K_{RBC/plasma}$), which depends on the permeability of the cell membrane for the analyte and the affinity of the analyte for constituents within the plasma and red blood cells.[253,254] As previously mentioned, TPM binds to carbonic anhydrase in the red blood cells in which TPM will have greater affinity to a constituent of red blood cells particularly at lower concentrations.[93] Hematocrit values reflect the amount of red blood cells in whole blood. This varies between individuals with ranges for different age groups presented in Table 22.[255]

Table 29: Normal Values for hematocrit per age

Age	Hematocrit (%) (± 2 SD)
Birth	51 (42-64)
<1 month	44 (31-67)
1-2 months	35 (28-55)
2-6 months	36 (28-42)
0.5- 2 years	36 (33-40)
2 to 6 years	37 (34-40)
6-12 years	40 (35-45)
Female	
12-18 years	41 (36-46)
> 18 years	41 (36-44)
Male	
12-18 years	43 (37-49)
> 18 years	47 (41-50)

The following equations relate the volume of whole blood to the volume of plasma and red blood cells, if the hematocrit is known.

$$V_P = (1 - Hct) * V_b \tag{6}$$

$$V_{RBC} = Hct * V_b \tag{7}$$

The red blood cell concentration can be calculated using the red blood cell-to-plasma partitioning and the concentration in plasma, equation 5.

$$C_{RBC} = K_{RBC/Plasma} * C_P \tag{8}$$

When substituting these equations into equation 2, plasma concentration can be calculated from whole blood or DBS.

$$C_P = \frac{C_b}{(1 - Hct) + K_{RBC/plasma} * Hct} \tag{9}$$

Plasma concentrations were calculated from the whole blood or DBS concentration using equation 9, where C_b is either whole blood or DBS. The average hematocrit for the population in children (0.40 L/L), adult males (0.47 L/L) or females (0.41 L/L) and the value for $K_{RBC/Plasma}$ were entered as fixed values for this equation.

Calculated plasma samples and analyzed plasma samples were compared using weighted Deming regression. The agreement between the methods were compared using the Bland-Altman plot, a statistical method to compare two measurement techniques in clinical chemistry.[256] This plot presents a graphical method in which the differences between the two techniques are plotted against the average of the difference of the two techniques. This method has previously been used in comparison of blood spot measurement in LC-MS and plasma in FPIA of TPM.[241] If the differences between measurements using the two assay methods lie within the limits of agreement of the Bland-Altman test 95% of the time, this indicates that the two methods are not producing different results. All statistical analyses were conducted with GraphPad (Prism 6.0, San Diego, CA). Acceptable criteria for the agreement between calculated and analyzed plasma concentrations were based on the guideline on Bioanalytical Method Validation of the FDA; the difference in concentration should be with $\pm$15% of their mean for at least 67% of the samples.[216]

5.4.3 Pharmacokinetic Analysis

Population PK studies can identify and quantify sources of variability in parameter estimates in the patient population. Samples can be collected from patients taking different doses over different periods of time. As extensive samples from one person are not required, a population approach is useful for investigating patient groups that are difficult to study, such as premature infants. The population PK approach will be used in analysis of the adult and children TPM concentration-time data.

Steady-State Clearance

The time of last dose and time of sample were recorded to determine the time after dose. Given TPM's long half-life and patients were on their maintance dosethe concentration measured was assumed to be close to the average steady state concentration. The steady-state clearance was calculated from the following equation for plasma and whole blood clearance and compared for each subject.

$$Cl = \frac{\left(\frac{Dose}{\tau}\right)}{C_{ss}} \tag{10}$$

Population Model

The PK parameters were estimated using Phoenix software (WinNonLin, Version 6.3; Pharsight Corporation, Mountain View, CA, USA). The first-order conditional estimation (FOCE) method produced estimates of the structural parameters, as well as estimates of inter-individual and residual unexplained variability for the PK parameters.

Model selection was based on the change in objective function value (OFV: for nested models), Akaike information criterion (AIC; for non-nested models), and the visual inspection of improvements in the diagnostic plots (observed vs. population and individual predicted concentrations and conditional weighted residuals vs. predicted concentration and time). For testing covariate models, a decrease in the OFV greater than 3.84 ($\alpha = 0.05$, df = 1) between two nested models was considered significant. Demographic and clinical variables tested as potential covariates were age, sex, and concomitant medications (PHT and CBZ) where concomitant medications were included together. The covariate model was built in a stepwise fashion with forward selection and backward deletion. Each covariate was added to the base model one at a time in the forward selection based on previously described model selection criteria. Covariates that did not increase the minimized OFV by more than 3.84 ($\alpha = 0.01$, df = 1) were deleted from the full model.

Both one- and two-compartment PK models with first-order absorption and elimination were tested to describe the plasma concentration–time profiles of TPM. The analysis utilized sparse sampling methods with one data point per subject. The time of sample collection and time of last dose were used to determine time after dose for each subject. The models were parameterized in terms of clearance (CL), volume of distribution (V), and first-order absorption rate constant (Ka). The between-subject variability (BSV) was estimated using a multiplicative error model and expressed as a coefficient of variation (CV). Plots of post hoc estimates of PK parameters from the base model versus covariates were visually inspected to evaluate the magnitude and direction of the

covariate effects. A standard forward inclusion–backward elimination approach was adopted for developing the covariate model. The covariates examined were age, sex, and comedication. Continuous covariates, such as age, were modeled through linear regression on clearance. The effect of categorical covariates (sex and comedication) was examined through a multiplicative model in order to obtain the fractional change in the PK parameters.

Because the available concentration data contained little information about the absorption phase, absorption rate constant (k_a) could not be estimated properly and was fixed at 2 h^{-1} as used in previous reports.[139,140,143] Additionally, the residual error was fixed at 15%.

5.5 RESULTS

Of the 38 recruited patients, 33 patients (adults: median age 36, range 21-55; children: median age 7, range 6-12) provided at least one usable spot and blood from a venipuncture. Table 30 describes the demographics.

Table 30: Demographics

Characteristics	Adults	Children
Number of subjects	30	8
Number of excluded DBS	5	0
Number of missing venipuncture	1	0
Male	14 (46.67%)	2 (25%)
Age, median (Range)	36 (21-55)	7 (6-12)
Males, median (Range)	40 (25-54)	
Females, median (Range)	35 (21-55)	
Hematocrit, median (Range)	38 (27-49)	35 (32-38)
Males, median (Range)	40 (33-49)	
Females, median (Range)	37 (27-41)	
Drug Monitoring (mcg/ml), mean (standard deviation)		
Mean plasma levels	9.95 (7.92)	7.56 (3.59)
Mean whole blood levels	11.33 (7.26)	7.72 (4.03)
Mean DBS levels	13.52 (5.26)	7.51 (4.01)
Average dose (mg/day), mean (standard deviation)	357 (214)	194 (80)
Range (mg/day)	50-1400	60-350
AED Concomitant Medications		
Enzyme Inducing (CBZ, PHT)	5	0
Neutral (LTG, VPA, None)	25	8

5.5.1 TPM Binding to Carbonic Anhydrase

Whole blood and plasma concentrations were shown to be different. Additionally, the whole blood-to-plasma ratio changed with plasma concentrations indicating that TPM is distributing into red blood cells. The distribution into red blood cells may be due to its binding to carbonic anhydrase isoforms that are present in high concentrations in the erythrocytes.[251] Figure 21 shows the whole blood/plasma concentration ratio for TPM as a function of the plasma concentration. The figure suggests there is greater binding at lower concentrations and becomes saturated as plasma concentration increases.

Binding analysis revealed that binding comprised a saturable component (Figure 21). When a single binding site was assumed, the optimal fit yielded a K_D of 0.5 mcg/ml (95% confidence interval, CI = 0.4-0.5) and a B_{max} of 6.3 mcg/ml (95% confidence interval, CI = 6.2-6.5). The two saturable binding site model was unable to fit the data and was over-parameterized given the small sample size.

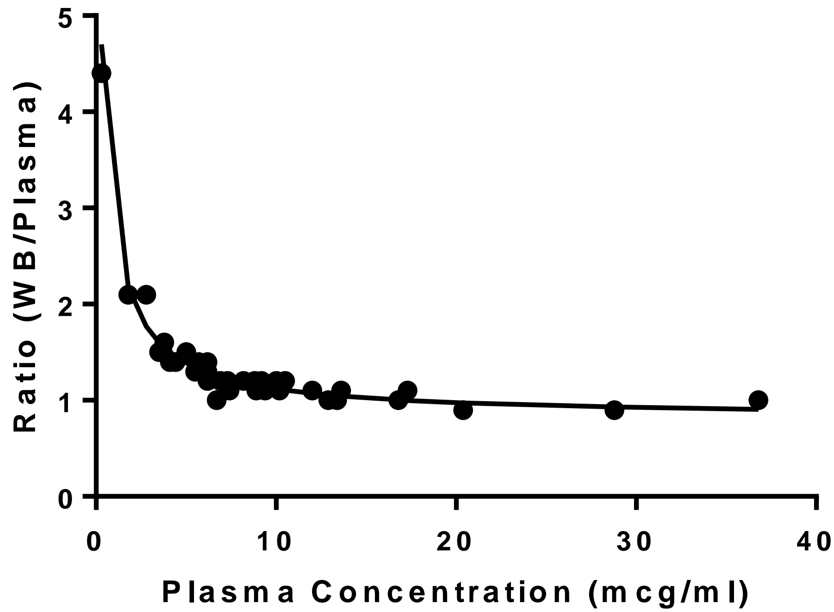


Figure 21: Plot of Whole Blood/Plasma Concentration Ratio for TPM as Function of the Plasma Concentrations. The curvilinear line formed by a curve fit analysis of the data assuming one saturable binding site.

Table 31: Final Parameter Estimates for Binding Model

Parameter	Units	Estimate	Std. Error	%CV
K_D	mcg/ml	0.5	0.04	8.6
B_{max}	mcg/ml	6.3	0.08	1.2
Random Unexplained Variability		5.9 %	0.01	21.5

5.5.2 Estimation of Plasma Concentrations

The analyzed concentration in blood was higher than the analyzed plasma concentration.

The red blood cell-to-plasma partitioning coefficient appears to change with

concentration. Thus, six different partitioning groups were formed based on concentration. The red blood cell-to-plasma partitioning coefficient was determined to be 12.2, 2.2, 1.5, 1.2, and 1.02 for whole blood concentration of 0-2.5 mcg/ml, 2.5-5 mcg/ml, 5-10 mcg/ml, 10-15 mcg/ml, and greater than 15 mcg/ml, respectively. The red blood cell-to-plasma partitioning coefficient was determined to be 17, 2.2, 1.6, 1.4, and 1.0 for DBS concentration of 0-5 mcg/ml, 5-10 mcg/ml, 10-15 mcg/ml, 15-20 mcg/ml, and greater than 20 mcg/ml, respectively. These values were established ex vivo using fresh human whole blood and calculating the RBC concentration in patients. The partitioning is different between whole blood and DBS at the lower blood concentrations because the DBS concentration was slightly higher than whole blood concentrations due to greater recovery in the analytical method.

Table 32: Red Blood Cell Partitioning

Type of Assay	Blood Concentration (mcg/ml)	RBC-to-Plasma Partitioning
Whole Blood	0-5	9.1
	5-10	1.6
	10-15	1.2
	> 15	1.0
DBS	0-5	17
	5-10	2.2
	10-15	1.6
	15-20	1.4
	>20	1.0

Plasma concentrations were calculated from whole blood or DBS using equation 9, where individual hematocrit and average hematocrit for the population in children (0.40 L/L), adult males (0.47 L/L) or females (0.41 L/L) were used as fixed values. The obtained $K_{RBC/plasma}$ (Table 32) from ex vivo and in vivo studies was used as fixed values given the

blood concentration. Weighted Deming regression was used to investigate the relationship between calculated plasma concentrations from whole blood and DBS and analyzed plasma samples. These relationships are shown in Figure 22.

For whole blood, the slope was 0.97 (95% CI, 0.93 – 1.01), and the intercept was 0.41 (95% CI, -0.04 – 0.86); and for DBS, the slope was 1.00 (95% CI, 0.94 – 1.06), and the intercept was -0.12 (95% CI, -0.76 – 0.52) when the population average hematocrit was used. Additionally, when individual hematocrit was used the slope was 0.97 (95% CI, 0.93 – 1.01), and the intercept was 0.56 (95% CI, 0.11 – 1.01) for whole blood; and for DBS, the slope was 1.06 (95% CI, 1.00 – 1.12), and the intercept was -0.29 (95% CI, -0.90 – 0.33).

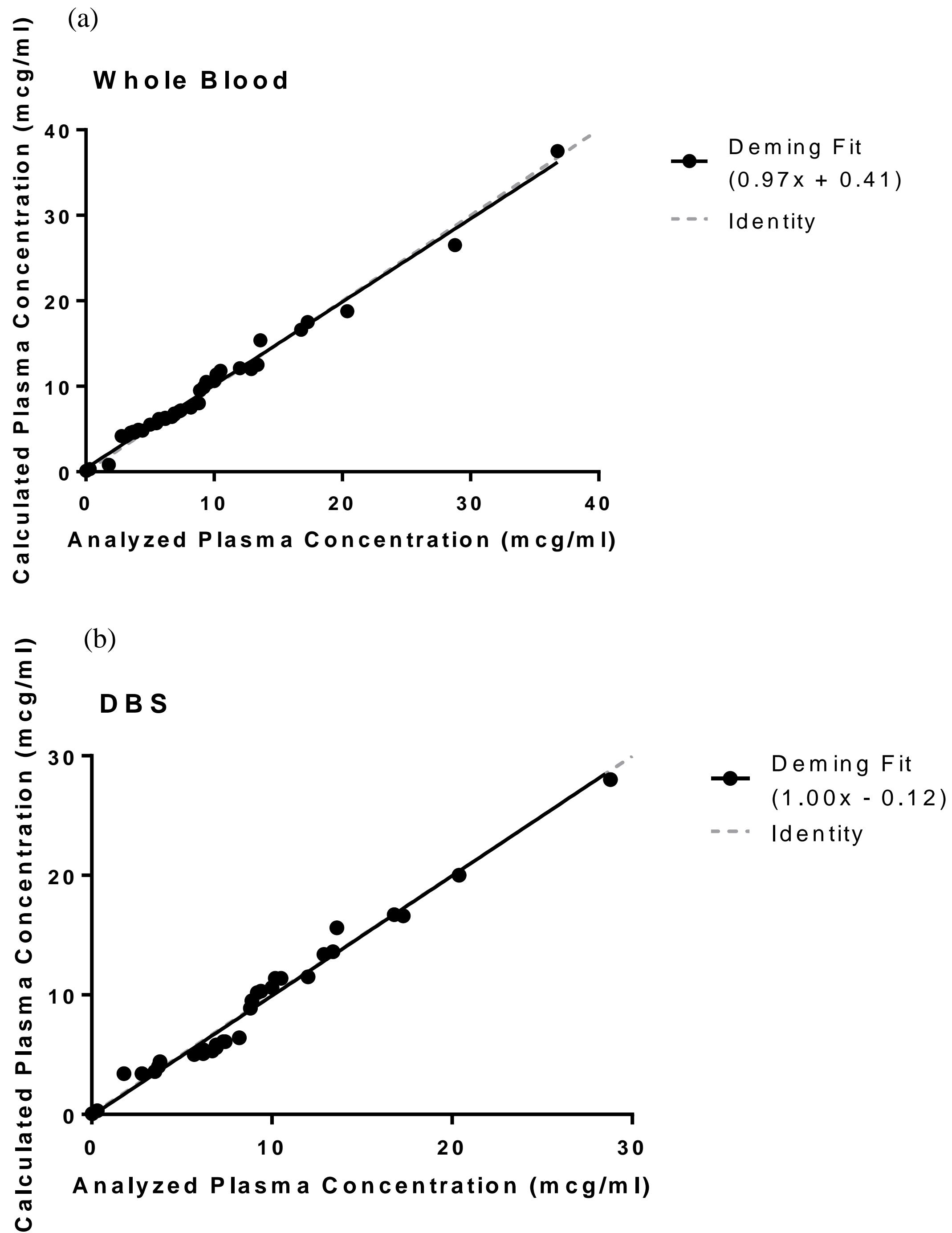


Figure 22: Calculated plasma concentrations, based on analyzed whole blood samples using average study hematocrit plotted against analyzed plasma concentrations (a) whole blood (N=37) (b) DBS (N=33).

The Bland-Altman difference plot (Figure 23) showed a small bias for the calculated plasma concentrations (WB: 0.16 mcg/ml, DBS: -0.09 mcg/ml) when using the average population hematocrit. The blank line is the line of true identity, the dashed line is the mean observed difference, whereas the dotted lines resemble a 95% bias agreement. Calculated plasma concentrations based on individual hematocrit resulted in a 0.27 mcg/ml and 0.25 mcg/ml biases for whole blood and DBS, respectively. Mean calculated plasma concentrations were within $\pm$ 15% of the mean of the analyzed plasma concentrations in 84% of the patient samples for whole blood and in 69% of the patient samples for DBS.

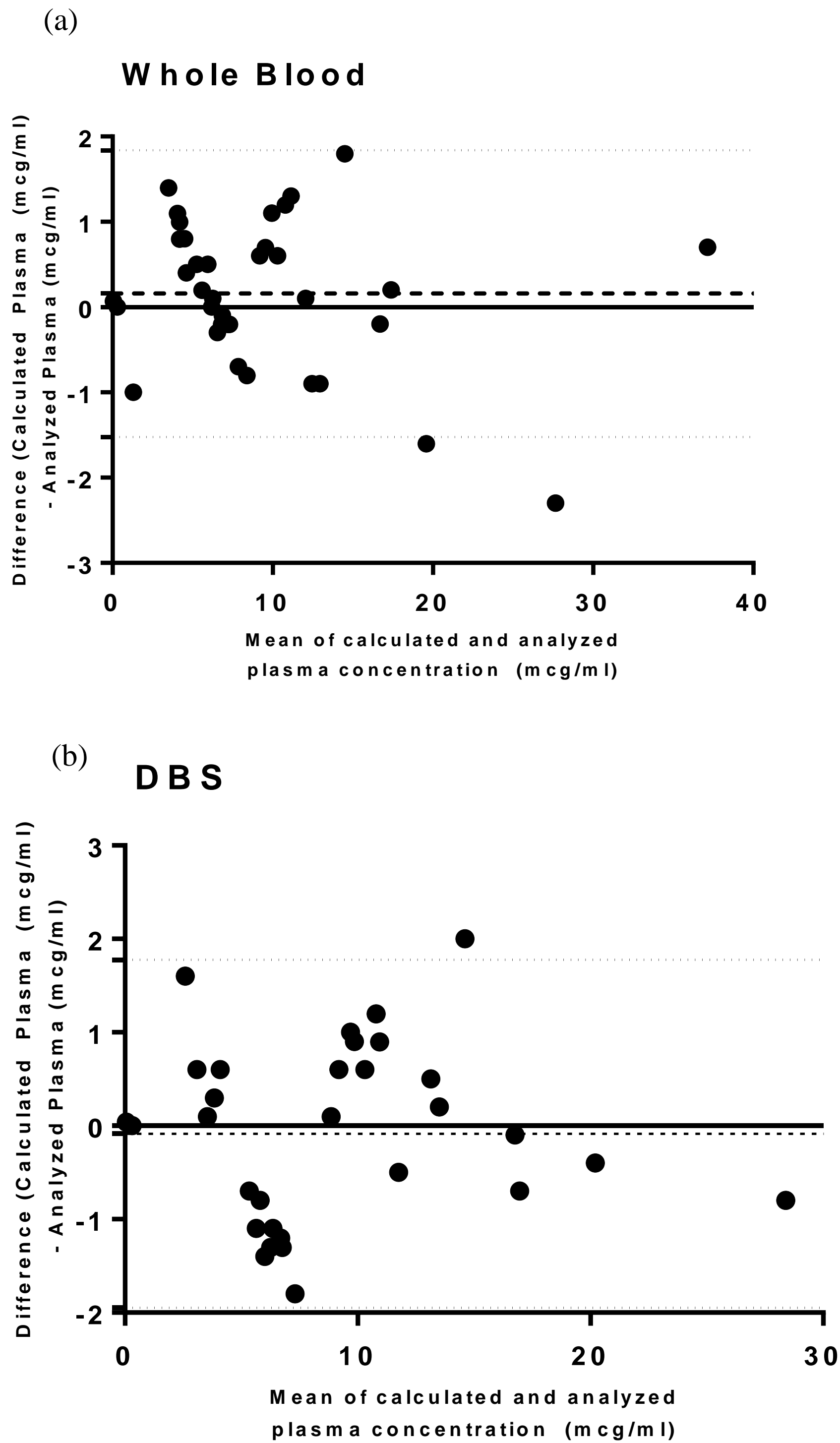


Figure 23: Bland-Altman plot for TPM using (a) whole blood (N=37) (b) DBS (N=33). Bold dashed lines indicate the bias and dashed lines indicate the 95% confidence interval

The fixed values for hematocrit and $K_{RBC/Plasma}$ can be imputed into equation 9. This results in a table (Table 33), which can be used to calculate plasma concentrations from whole blood concentrations.

Table 33: Correction Values for Whole Blood TPM Concentrations Based on Age Group and Gender

Group	Whole Blood Concentration (mcg/ml)			
	0-5	**5-10**	**10-15**	**>15**
Adult				
Male	4.81	1.28	1.09	1.00
Female	4.32	1.25	1.08	1.00
Children	4.24	1.24	1.08	1.00

5.5.3 Pharmacokinetic Analysis

Steady-State Clearance

The distribution of TPM clearance for whole blood and plasma are shown in Figure 24. The mean clearance for plasma was 1.96 L/hr (SD=1.74) compared to 1.52 L/hr (SD=1.13) for whole blood. Using a paired t-test, there was a significant difference between whole blood clearance and plasma clearance (p-value = 0.001).

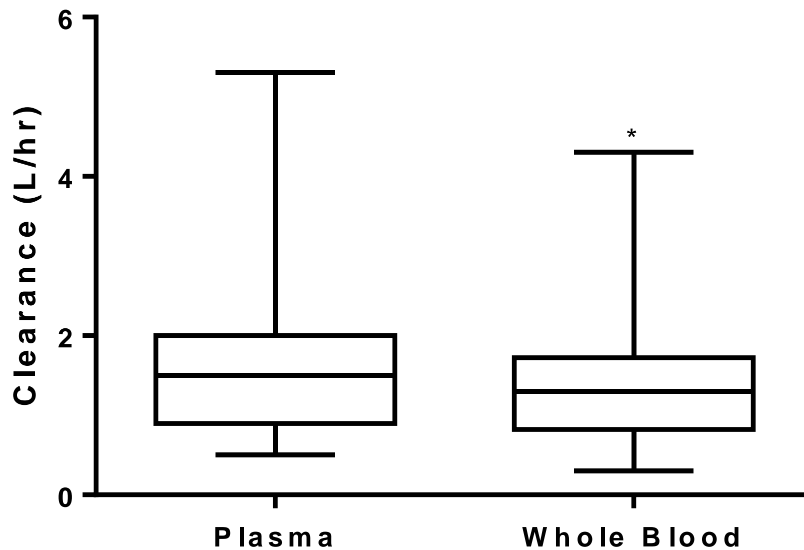


Figure 24: Distribution of Plasma and Whole Blood Clearance. Box-plot showing minimum to maximum clearance (N=37).

In addition to the whole blood and plasma clearance, comedication effect on plasma clearance was explored. The plasma clearance of TPM ranged from 0.5 L/hr to 5.3 L/hr. When patients were taking neutral AEDs the mean plasma clearance was 1.42 L/hr. Conversely, patients taking enzyme inducers showed mean plasma clearances of 3.52 L/hr, respectively. Inducing medication use did significantly alter clearance determined by a one-way ANOVA ($p<0.0001$). The distribution of TPM plasma clearance based on comedication is displayed in Figure 25.

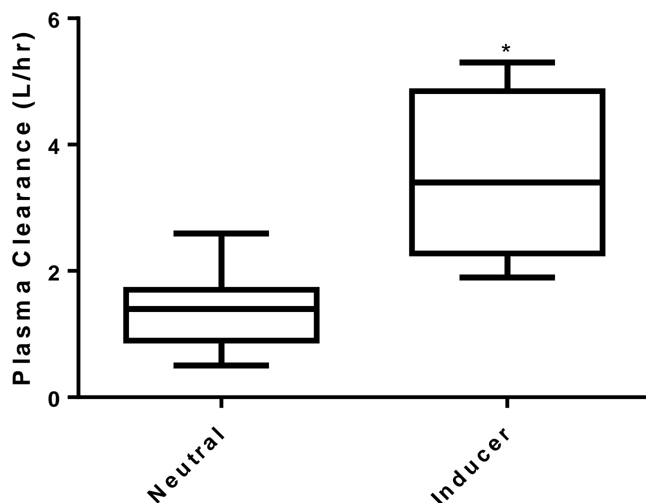


Figure 25: Distribution of Plasma Clearance Based on Comedication. Box-plot showing minimum to maximum clearance (N=37).

Population Model

Final PK model parameter estimates and goodness-of-fit plots are presented in Table 34 and Figure 26, respectively. The final PK model was a one-compartment model with first-order absorption and elimination and a multiplicative model for BSV of CL/F. During the forward inclusion, only comedication showed a significant influence on the CL/F (ΔOFV = 209.91 and 199.5, respectively, $P < 0.01$). A two-compartment model was unable to fit the data. This is likely because of the small sample size and lack of samples at the early time points.

Covariate analysis showed that concomitant medications with CBZ and PHT had a significant effect on clearance. The patients with the highest clearance were on at least one inducing antiepileptic drug (carbamazepine or phenytoin). Age and sex had no effect on clearance.

Table 34: Parameter estimates for final pharmacokinetic model

Parameter	Estimate (%RSE)	95% CI	BSV (%RSE)
CL/F (L/hr)	1.43 (11.4)	(1.1,1.8)	30.8% (18.7)
Effect of Inducer	0.82 (23.3)	(0.42,1.21)	
V/F (L)	20.79 (27.5)	(8.3,33.87)	NE
K_a (h^{-1})	Fixed at 2		
Residual error (%)		Fixed at 15%	

*RSE, relative standard error = (standard error ÷ estimate) × 100, NE, not evaluated; 95% CI from 500 bootstrap estimates; BSV, between-subject variability

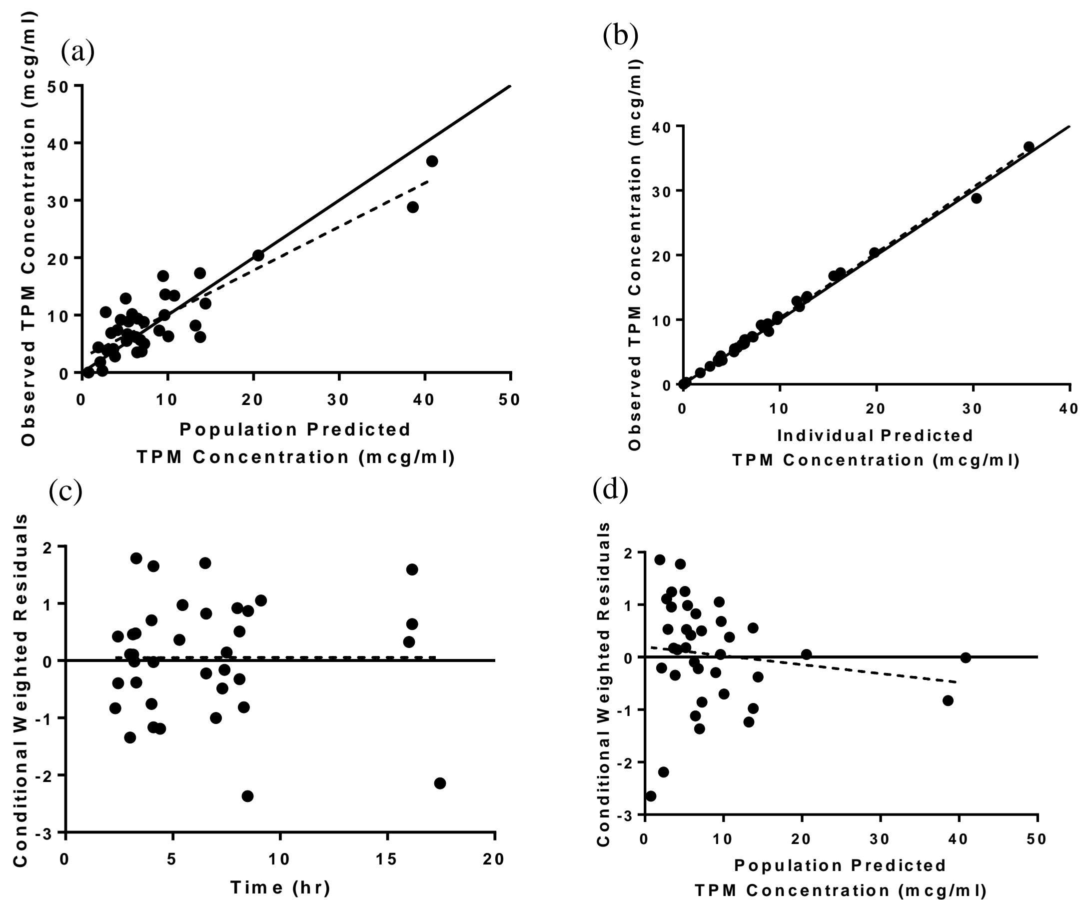


Figure 26: Goodness-of-fit plots from the final PK model. (a) Identity plots of observed vs. population predicated TPM concentration. (b) Identity plot of observed vs. individual predicted TPM concentration. (c) Scatter plot of conditional weighted residuals (CWRES) vs. time. (d) Scatter plot of CWRES vs. population predicted TPM concentration. Line of identity (solid); regression line (dashed)

Patients taking inducers have on average a larger or smaller clearance than those not taking inducing medications (see equation below).

Clearance (L/hr) = 1.43 + 0.82 (if taking inducer)

A sensitivity analysis was performed for the final model to determine the impact of the modeling assumptions (fixed k_a) on the resulting structural and covariate parameter estimates. The population PK model was fitted to the TPM data and values for k_a were fixed to represent absorption half-lives of 0.23 to 0.69 hours. This represented a range of plausible absorption times. Results from the sensitivity analysis for k_a are shown in Table 35. CL/F estimates ranged from 1.47 to 1.53 L/hr, and V ranged from 20.28 to 21.10 L over the range of k_a values examined. The values represent a <5% difference from the final model parameter estimates.

Table 35: Sensitivity analysis results for k_a

Absorption $t_{1/2}$ (hr)	**K_a (hr^{-1})**	**CL/F (L/hr)**	**V/F (L)**	**Inducing on CL**	**BSV_{CL}**
0.69	1	1.53	20.28	0.85	0.316
0.46	1.5	1.50	21.04	0.83	0.311
0.35	2	1.48	21.10	0.82	0.306
0.28	2.5	1.48	21.03	0.81	0.302
0.23	3	1.47	20.97	0.80	0.299

5.6 DISCUSSION

This study examined the relationship between whole blood and plasma TPM concentrations and characterized the PK. Calculated TPM plasma concentration based on whole blood and DBS concentrations are in good agreement with analyzed plasma concentrations. We showed that the difference between concentrations in whole blood or DBS and plasma can be explained well by the analyte-specific RBC-to-plasma partitioning and hematocrit using a formula from the literature.[253] For whole blood and DBS, this formula resulted in a very small bias between calculated and analyzed plasma concentrations, only 0.16 mcg/ml and -0.09 mcg/ml, respectively. Furthermore, 84% and 69% of the calculated plasma concentrations in patients samples were within 15% of the analyzed plasma concentrations; we believe these biases are acceptable. Additionally, in a clinical setting a 1-3 mcg/ml difference would be acceptable for clinical practice.

The RBC-to-plasma partitioning was found to be dependent on the concentration. Therefore, groups were developed to be imputed as a fixed value across a concentration range. Our target population consisted of adults and children receiving TPM treatment. The hematocrit variation between individual and population average was examined. Using each of these values, it was found that the biases ranged from 0.16 - 0.27 mcg/ml. The small biases indicate that for this application, it may not be necessary to impute patient-specific hematocrit values in the conversion formula and the average hematocrit for male, female, and children could be imputed as a fixed value. This finding is important since hematocrit determination requires a venipuncture and would therefore diminish the large advantage of DBS sampling. When using fixed values for both RBC-

to-plasma partitioning and hematocrit, a table for the conversion of DBS/whole blood concentration to plasma concentration were created for adult males and females and children (values shown in Table 33). This method provides a simple correction value for physicians or laboratory personnel. Additionally, it appears that individual hematocrit (if available) and average hematocrit for the population can be used in the equations without significantly changing the calculated plasma concentration. The hematocrit range for the patients in our study was 27 – 49%. We did not find a correlation between whole blood concentration and hematocrit. This could be because of the small range of hematocrit values in the patient population in this study. Further studies would need to be performed to further examine the effect of hematocrit on whole blood concentration especially at younger ages when hematocrit is higher than adult values.

This is the first study comparing TPM plasma and DBS concentration obtained from patient samples using a formula that includes both hematocrit and the analyte-specific red blood cell-to-plasma ratio. This is especially interesting because the TPM RBC-to-plasma partitioning coefficient changes with concentration which has not been calculated previously. This study also showed that a 3 mm punch sample on a DBS card can be used to determine TPM concentrations in patients. For most subjects in our study at least one usable DBS was obtained. In the future, with proper training, patients could use this technique in order to keep the number of unusable spots to a minimum.

In addition to the calculation of whole blood to plasma TPM concentrations, the binding of TPM to carbonic anhydrase was explored. In our study (assuming one saturable site),

the B_{max} and K_D values was 6.3 and 0.5 mcg/ml, respectively. These values are in good agreement of reported values for a one-site saturable binding model reported previously.[93]

The mean clearance was 1.43 L/hr (SD= 1.10) in adults and children and was significantly affected by inducing comedication use. Approximately 20% of TPM is metabolized when administered in the absence of enzyme inducers.[94] When TPM is administered with an enzyme inducer, the metabolic clearance increases and up to 50% of the dose undergoes metabolism.[80,98,99] The current study found a 5-fold range of clearance, from 0.5 L/hr to 5.3 L/hr. The estimated TPM clearance obtained in this study is very similar to those obtained in previous studies.[91,93] The narrow ranges of time after dose and concentrations in this study were limitations to investigating the population PK. These limitations result in an unreliable model for the estimation of volume of distribution. Additionally, there was only one sample per subject which limits the intra-subject variability that can be estimated.

In the current study, a significant difference was observed between TPM clearance calculated from blood and plasma data. There was a larger difference between plasma and whole blood clearances at lower concentrations. This may be a result from a larger percentage of TPM being bound to carbonic anhydrase at lower concentrations and during drug elimination the release from these saturable binding sites contributes to reduced clearances for blood at low doses. The volume of distribution was found to be lower than previous reported values. As the 95% confidence interval obtained by

bootstrap method was extremely large it is likely that we could not obtain a good estimate of volume based on the limited data.

The small sample size is a major limitation of the study and future studies need to confirm these results and further explore the hematocrit at younger ages.

CHAPTER 6: CONCLUSIONS

TPM is widely used in the treatment of epilepsy and migraines and is effective in controlling seizures in children and adults. TPM has the potential to be an effective therapy of neonatal seizures and Dravet syndrome as discussed in Chapter 2. One major hurdle in developing drugs for treatment of childhood seizure disorders is the severe restriction on blood sampling in critically ill children particularly infants and neonates, from whom blood is collected for a multitude of lab tests. This circumstance reduces the blood volume available for PK studies. Consequently, the necessary research on PK and dose finding is often significantly limited.

One solution to this problem would be to use whole blood or DBS to measure drug concentrations in place of plasma. This approach will reduce the volume of blood needed for a drug assay by greater than 75%. Complicating the use of whole blood for TPM measurements is the fact that the drug exhibits saturable binding to carbonic anhydrases in RBCs. As a result, there is a non-linear relationship between whole blood and plasma, which greatly complicates interpretation and comparison of TPM concentrations.[93]

If TPM is measured in whole blood without correction for non-linear binding this would result in inaccurate PK estimates. For example, Filippi et al used DBS to measure TPM concentrations in neonates.[109] Blood values were corrected according to the patient's hematocrit to obtain plasma values using an equation that assumed TPM is isolated in the plasma. This will result in concentrations greater than what would be seen if properly correcting for TPM binding to RBCs. The important aspect of this study is that this is the first report of TPM PK in newborns. So, if you are trying to determine a dosing regimen

presenting these misleading concentrations could result in clinicians under dosing patients. It is also important to establish this relationship between whole blood and plasma concentrations to correctly assess TPM PK and to establish a therapeutic range.

With a DBS assay we could measure TPM concentrations in children while limiting the volume of blood that is collected. The aim of this thesis research was to develop a sensitive and selective bioanalytical assay to quantify whole blood and DBS TPM concentrations, use the assay to measure whole blood in dogs and whole blood and DBS in adults and pediatrics, and implement these assays in clinical practice. The results were then used to characterize the relationship between whole blood and plasma TPM concentrations.

The availability of reliable and sensitive bioanalytical methods is a prerequisite in the determination of drug exposure in patients. An HPLC-MS assay using plasma, whole blood, and DBS was developed according to FDA guidelines. In particular, the DBS assay showed good performance on accuracy and precision. TPM was shown to be stable in DBS when stored at -20°C for 6 months. Whole blood and DBS concentrations are usually not similar to plasma concentrations, due to differences in analyte-specific red blood cell-to-plasma partitioning and patient-specific hematocrit. The studies described in this thesis showed that the difference between whole blood and plasma can be explained using equation 9 (Chapter 5, page 123). The red blood cell-to-plasma partitioning coefficient was found to be concentration dependent. Therefore, groups based on whole blood or DBS concentration were formed and imputed as fixed values in

the equation. Also, it was determined that hematocrit could be imputed as a fixed value depending on gender and age group. When using fixed values for both red blood cell-to-plasma partitioning and hematocrit, a simple table (Table 33) for the conversion of DBS concentrations to plasma concentrations was obtained.

In the canine study, both intravenous and oral TPM were administered with the goal of comparing the PK of intravenous TPM in plasma and whole blood and describing the relationship between whole blood and plasma concentrations in dogs with naturally-occurring epilepsy. Whole blood and plasma samples were obtained at predetermined times up to 8 hours post-dose. Similar to humans, the study showed a nonlinear relationship between plasma and whole blood TPM concentration in which whole blood TPM concentrations were 1.3-3.7 times greater than plasma concentrations. Caution should be exercised in interpreting whole blood TPM concentrations as one may overestimate plasma concentrations by as much as 4 fold. There was a difference in PK parameter estimates with those derived from whole blood generally showing reduced clearances resulting in longer elimination half-lives. This difference is likely because during drug elimination, the release of TPM from high-affinity saturable binding sites in erythrocytes following low doses may contribute to the reduced clearance. Future studies of TPM in other laboratory animals and dogs are needed to verify this relationship between plasma and whole blood.

Assuming the rapid distribution of TPM from plasma to other body compartments includes the CNS, IV TPM may be useful in treating canine seizure emergencies and may

have potential use in human seizure emergencies. Further study is needed on the use of IV TPM for seizure emergencies.

In the adult and pediatric study, whole blood concentrations were found to be different from plasma concentrations. This also led to a nonlinear relationship particularly at low concentrations. These are the first studies comparing plasma and DBS concentration obtained from patient samples using a formula that includes both hematocrit and the analyte-specific red blood cell-to-plasma ratio. In addition to the calculation of whole blood to plasma TPM concentrations, the binding of TPM to carbonic anhydrase was explored. In our study (assuming one saturable site), the B_{max} and K_D values was 6.3 and 0.5 mcg/ml, respectively. These values are in good agreement with reported values for a one site saturable binding model reported previously.[93] However, our sample size was small.

The plasma and whole blood clearances are similar to previous reported values. This further validates the plasma and whole blood assay for determination of TPM concentrations. Further clinical research to investigate the value of TDM using DBS is warranted. DBS can possibly be used by patients to self-sample at home and no special conditions for transport or storage are required. It would be possible, in theory, to obtain a DBS sample on a regular basis. This could provide information about compliance and individual differences.

In conclusion, the research described in this thesis shows that a whole blood and a DBS spot assay can be used to measure TPM concentration if the necessary corrections are used.

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